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516. A SIMPLE METHOD FOR PREPARING CRYSTALLINE RENNIN

BY N. J. BERRIDGE AND CORA WOODWARD

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The preparation of crystalline rennin previously described (1) was somewhat long and inconvenient, but the knowledge gained about the properties of the crystals suggested that a simpler method might be successful. Since then Møgensen (2) has shown that the mere precipitation of commercial rennet with saturated sodium chloride gives a product with proteolytic properties resembling those of the crystalline material, and with enhanced milk-clotting activity per unit of dry weight. Some years previously, Hankinson (3) had also achieved considerable purification by these means. The results of an attempt to discover a simple method of crystallization are described below.

METHODS

The coagulating power of preparations was measured in reconstituted spray-dried skim milk made N/50 with respect to calcium chloride as previously described (1). Details are given by Berridge (4).

PREPARATIONS

(1) The following example is the best of four successful preparations. The others behaved in slightly different ways, which are also described below. Throughout the process all solutions were maintained saturated with thymol.

One gallon (4·5 l.) of commercial rennet (from Bengers Ltd., Holmes Chapel, Cheshire) was saturated with sodium chloride. The supernatant liquid was decanted on to large fluted filters made from Whatman no. 3 paper, supported at the tip with a small no. 54 paper. The first opalescent runnings were returned to the filters. Four days were required for the filtration. After this time the papers had become partly dried, so that they were impregnated with solid sodium chloride. Moreover, the precipitated proteins were entrained in the filters and could not be removed by scraping. The no. 3 papers were therefore pulped and extracted exhaustively with distilled water. This required four extractions of approximately 250 ml. each. The combined extracts were filtered (Whatman no. 1), adjusted to pH 5·4, and again saturated with sodium chloride. This time the sodium chloride was added through a rotating, semi-permeable membrane (Visking tubing), as suggested by McMeekin (5).

By this technique, 2 days were required for the outer protein solution to reach saturation. Owing to the slow addition of the salt, the protein formed a granular precipitate which was easily centrifuged down. It was redissolved in 60 ml. of distilled water and, on standing overnight in the refrigerator, gave a good crop of crystals. The yields of enzyme at various stages are shown in Table 1.

(2) This was carried out as no. (1) until the stage of crystallization. Here, however, the solution was only half the concentration of the corresponding solution in no. (1), and sodium chloride was added very slowly through a rotating cellophane U-tube, 7 mm. in

diameter, dipping just below the surface. Addition was stopped after a few hours when the solution had become slightly opalescent. Crystallization occurred after 4 days in the refrigerator.

Table 1. *Yield of rennin at different stages of separation*

Solution	Total acitivity (% raw material)
Commercial rennet, 1 gallon	100
Combined filter extracts	55
Solution of granular precipitate	43
Suspension of crystals	18
Crystals after dialysis	14*
Solution of crystals after centrifuging	12*
Suspension of recrystallized rennin (second method)	5*

* These figures are twice the experimental values, since only half the material was taken for this recrystallization.

(3) This behaved exactly as no. (2).

(4) For this, another brand of commercial rennet was tried. The procedure was similar to no. (2) in essentials, but an extra precipitation and re-solution was used. Finally, two active precipitates and an active supernatant were obtained by proceeding a little further with the final slow addition of salt and centrifuging at suitable stages, as judged by the turbidity of the solution. The precipitates were re-dissolved in 2 and 10 ml. of water respectively, and all three fractions, containing *in toto* 30% of the original activity, deposited crystals after a fortnight in the refrigerator.

Recrystallization

Half of the crystals from preparation no. (1) were suspended in 15 ml. of 0·1 M-acetate buffer, pH 5·4, and dialysed against repeated changes of distilled water for 2 days. As solution was not complete, the precipitate was separated by centrifuging and extracted twice with 10 ml. of distilled water. The second extract, being cloudy and of low activity, was treated separately. The first was added to the main solution and the mixture was warmed to 37° C. to dissolve an amorphous precipitate which had appeared on storing in the refrigerator. Traces of insoluble material were centrifuged off, and the clear solution set aside to crystallize at room temperature, with the addition of 0·5 g. of NaCl every 2 days to diminish the solubility of the rennin. Crystallization began after the second addition, and was complete after 4 more days. There was a loss on recrystallization of about 50%.

The second half of the crystals was dialysed and extracted with water similarly, but the solution was precipitated by saturating with sodium chloride. The resulting amorphous precipitate was centrifuged down and re-dissolved in the minimum volume (30 ml.) of 0·1 M-acetate buffer at pH 5·4. Crystallization began almost at once. Here again losses were large, as may be seen from Table 1. The causes of this loss have not yet been investigated.

DISCUSSION

The simplicity of the present method compared with the first one (1) calls for some explanation. It is now known that the solubility of rennin crystals in salt solutions is very low, and it is thus possible to choose solutions less highly supersaturated from which crystallization, rather than deposition in the amorphous form, will take place. Another factor enabling the simplification to be made is the greater purity of the commercial rennet

now available. Improvements have been made in the collection of glands, which now reach the manufacturers in a fresher condition, enabling them to produce an enzyme solution containing fewer products of autolysis and bacterial degradation.

The simplicity of the preparation should facilitate those enzymological studies in which rennin may be used as the enzyme, as well as research more closely related to the clotting of milk and the particular properties of rennin itself. Furthermore, it should now be possible to use the crystalline enzyme as a standard of activity for the control of other preparations in research and industry. It is worth emphasizing that the yield of crystals first appearing is such as to make the preparation feasible on a larger scale, particularly since much of the enzyme not crystallizing is not actually lost but may be recovered from the filtrate and mother liquors in a form suitable for normal commercial use. Crystalline rennin may therefore be used in all experiments in cheese-making in which it is desirable to avoid the unknown effects of the other, possibly numerous, enzymes in ordinary rennet.

Since this work was done, our attention has been drawn to a paper by Connors, De Baun & Sullivan (6), read before the American Chemical Society. These workers have also prepared crystalline rennin and measured some of its properties, but their method of preparation has not yet been published.

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517. REACTIVATION OF MILK PHOSPHATASE FOLLOWING HEAT TREATMENT. II.

By R. C. WRIGHT and J. TRAMER
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(With 2 Figures)

In our previous paper on this subject (1), it was shown that when raw milk was rapidly heated to high temperatures, cooled and then stored at 30° C., a positive phosphatase reaction developed, and it was further shown that this phosphatase was identical with the alkaline phosphatase of raw milk, and was not of bacterial origin. When tested immediately after the heat treatment, such milks always gave a negative phosphatase reaction.

In the course of this work it was found that considerable variation occurred in the degree of reactivation with different milks. It was shown that this was not due to variations associated with technique, and from examination of a number of milks it was concluded that this variation was related to the milk itself.

Examination of possible factors affecting reactivation

In an attempt to elucidate the cause of this variation in reactivation a number of possible factors was examined, using the capillary technique previously described. Unless otherwise stated, shock treatment of raw milk means immersion of capillaries in a glycerine bath at 135° C. for 20 sec.; the subsequent storage period means 2 days at 30° C. Reactivation is expressed as parts per million chlorine, since the permanent colour standards used in the micro-phosphatase test (1) are calibrated in these units.

(*a*) *Fat content*

No correlation was found between the fat content and the level of reactivation within the range of fat content found in bulk raw milk from rail tanks. It had also been noted that marked reactivation had been obtained with both separated milk and milk rich in fat, though separated milk gave less reactivation than the corresponding whole milk.

(*b*) *Ascorbic acid*

The effect of adding ascorbic acid to the raw milk prior to shock treatment was examined, since in our previous experiments with a bacterial phosphatase—236—it appeared that reducing conditions might play a part. No effect upon the level of reactivation occurred on addition of ascorbic acid at the rate of 2 mg./100 ml.

(*c*) *Metallic ions and amino-acids*

Since it has been found (2) that either metallic ions such as Ca^{2+}, Mg^{2+} Zn^{2+}, Mn^{2+}, or amino-acids such as alanine, or both, play a role in the reactivation of alkaline phosphatase of intestinal origin, experiments were made to see whether Ca^{2+} or Mg^{2+} or alanine affected the reactivation of milk phosphatase, but no effect was observed using concentrations likely to occur as contaminants.

The effect of the presence of copper has also been examined, since reducing conditions seemed to be concerned with reactivation. No effect was observed in the presence of Cu^{2+} up to a concentration of 1 p.p.m., but reactivation was inhibited at a level of 5 p.p.m. as shown by the absence of reactivation when this concentration of Cu^{2+} was added to the milk either before or after shock treatment. A control experiment using raw milk phosphatase showed that this concentration of Cu^{2+} did not affect the hydrolysis of the buffer substrate. Thus variations in reactivation levels found in different raw milks cannot be due to slight differences in Cu^{2+} content.

(*d*) *The phosphatase content of raw milk*

Although in the majority of our experiments bulk raw milk from rail tanks was used, the possibility existed that the variations in phosphatase content of such milk, which in our experience may vary from one half to twice the normal amount, might be responsible, at least in part, for the variations encountered. Examination of a number of tank milks, however, showed that the level of reactivation was not related to the initial phosphatase content of the raw milk.

(*e*) *Effect of storage of raw milk*

During a few days when the atmospheric temperature was very high and the raw milk used was left standing in the laboratory during the day, it was observed that the level of reactivation obtained following shock treatment was higher in the afternoon than in the morning. This observation was followed up and the experiments made are described below.

Bacteriological quality of the raw milk and level of reactivation

Typical figures for reactivation obtained on raw milk allowed to stand at room temperature during a period of high atmospheric shade temperature are given in Table 1.

Table 1

	Shock treatment applied	
Milk no.	Morning (11–12 noon)	Afternoon (4–5 p.m.)
1	Trace	0·45
2	0·25	0·45
3	0·2⁻	0·5

This effect of incubation of the raw milk on reactivation was then examined more closely. Raw milk from a rail tank was shocked immediately after arrival in the laboratory and also after incubation at 37° C. and at atmospheric temperature for varying times. A control sample was left in the refrigerator.

The results given in Table 2 show the effect of incubation of the raw milk at different temperatures upon reactivation.

Table 2

	Incubation temperature		
No. of hr. incubation	4° C.	20° C.	37° C.
1½	0·2+	0·3+	0·4
2½	0·3	0·4+	0·6+
5½	0·4	0·5	Sour

From these results it will be seen that even when left in the cold store the level of reactivation increased, but though no figures were obtained the milk was almost certainly of only moderate bacteriological quality.

Subsequent tests showed that raw milks of poor bacteriological quality, as judged by the resazurin test, invariably gave good reactivation. On the other hand, it was noted that good quality raw milks gave variable reactivation—some milks giving a figure of 0·1 or less, whilst others gave the more normal figure of 0·2–0·4.

The influence of growing organisms was also shown by the effect of preheating the raw milk to 71° C. for 2 min. in capillaries before shocking, as can be seen from Table 3.

Table 3

Time				
Capillaries filled	Preheating 71° C./2 min.	Shocked 135° C., 20 sec.	Incubation room temp.	Reactivation
10.45 a.m.	—	11 a.m.	—	0·1⁻
10.45 a.m.	11 a.m.	11.05 a.m.	—	0·1⁻
10.45 a.m.	—	4.25 p.m.	5½ hr.	0·45
10.45 a.m.	11 a.m.	4.25 p.m.	5½ hr.	0·1
10.45 a.m.	4.25 p.m.	4.30 p.m.	5½ hr.	0·4

These results showed that the increase in reactivation which occurred when raw milk was incubated for several hours at room temperature did not occur if the raw milk was first preheated so as to destroy non-heat-resistant organisms. They further showed that the preheating itself, i.e. apart from its action on bacteria, did not appreciably affect reactivation, and accordingly it was concluded that the increase in reactivation which often occurs when raw milk is aged is due to the influence of bacteria. It was also concluded that many of the variations in reactivation encountered in the earlier work were due to this cause.

Many papers have been published on the role of growing bacteria in milk with particular reference to dye tests used to assess its bacteriological quality, and much of this work has been summarized by Wilson *et al.* (3). In this summary it is stated that the growth of bacteria in milk results in the utilization of the dissolved oxygen together with the production in the milk of reducing systems.

The influence of these two factors on reactivation was therefore examined.

Influence of oxygen

A number of raw milks of varying bacteriological quality was examined using the normal capillary technique. In some cases the milks were aged by incubation at various temperatures. Each milk was divided into portions, nitrogen bubbled through them and capillaries prepared immediately afterwards, one portion being left untreated.

From these experiments the following facts were found:

(1) With good quality milks removal of oxygen by passage of nitrogen usually led to a marked increase in reactivation.

(2) With poor quality milks, which gave good reactivation, removal of oxygen did not lead to any marked increase in reactivation.

These two observations applied equally to different milks and to the same milk allowed to deteriorate by suitable storage conditions. At the same time it was noted that some good quality milks gave good reactivation when untreated.

Table 4 shows a typical result obtained with two different milks.

Table 4

Time of bubbling nitrogen through milk (min.)	Reactivation: Good quality milk (10 min. resazurin 6) (30 min. reseazurin 5½)	Reactivation: Poor quality milk (10 min. resazurin 2) (30 min. resazurin 0)
0	0·15	0·45
15	0·3	0·5⁻
30	0·4⁻	0·55
60	0·4⁻	0·5⁺

Similar results were obtained when small test tubes measuring 10 × 0·75 cm. (F/D tubes), as used for freeze-drying bacterial cultures, were used. In certain experiments which involved a larger volume of milk than could be conveniently obtained by use of capillaries, 0·5 ml. of raw milk was shocked in these sealed tubes, and it was observed that the level of reactivation was much lower than when the same milk was shocked in capillaries. On removal of oxygen from such tubes, by bubbling through nitrogen immediately prior to sealing, greater reactivation was obtained. Examples of results obtained are given in Table 5.

Table 5. *Milks shocked at* 135° C./20 *sec. in capillaries and at* 135° C./45 *sec. in F/D tubes stored for* 3 *days at* 30° C.

	10 min. resazurin test reading	Reactivation: Capillaries	Reactivation: F/D tubes	Reactivation: F/D tubes + nitrogen
Good quality milk	5½	0·1⁻	Trace	0·35
	4½	0·25	0·1⁻	0·45
Poor quality milk	2	0·5	0·1⁺	0·3⁺
	½	0·6⁺	—	0·4⁺

It is realized that the conditions of heating are different in capillaries and small test-tubes of the above dimensions, but it is felt that the figures in Table 5 point to oxygen as being a major cause of the difference in reactivation obtained in capillaries and F/D tubes.

This marked effect of oxygen on reactivation has been confirmed on several occasions by comparing the results obtained on the milk as received, and the same milk in an atmosphere of nitrogen and oxygen respectively. The reactivation produced by shocking capillaries of milk saturated with oxygen was always less and often markedly so, than that given by the control milk. A similar effect, but to a less degree, has been found when the air space in the capillaries was increased.

Some experiments have also been made on the effect of the removal of oxygen on the reactivation of the bacterial phosphatase (236) in the presence of sterilized milk. When mixtures of aged broth cultures of this organism and sterilized milk were shocked in the absence of oxygen, increased reactivation occurred, but no reactivation of this phosphatase was obtained with pasteurized milk either in the presence or absence of oxygen.

Influence of reducing systems in milk

The influence of reducing systems on reactivation was first observed in the experiments made on a phosphatase of bacterial origin (236)(1). Reactivation of this enzyme was brought about by shock treatment of an aged broth culture in the presence of sterilized milk whereas no reactivation occurred in the presence of pasteurized milk.

The further experiments carried out on this aspect of reactivation and using this enzyme are described below.

Heated milks

Using mixtures of equal volumes of aged broth cultures of 236 and milk, a comparison has been made of the effect of different heat treatment of the milk upon reactivation of this bacterial phosphatase. It was found that maximum reactivation occurred using autoclaved milk (10 lb./15 min.). Autoclaving at a higher pressure and for a longer time gave somewhat lower values. Milk heated in a steamer gave lower values in direct proportion to the time of steaming.

Milk powder

Preliminary tests showed that reactivation of the phosphatase of 236 could be brought about by shock treatment of aged broth cultures containing added milk powder. The amount of milk powder added was found to have a considerable influence on the reactivation level, and accordingly the amount used was fixed at 0·1 g./ml. of broth culture. Various milk powders produced different levels of reactivation and subsequent experiments on powders of known origin and manufacture, showed that the reactivation produced was related to the milk preheating temperature. No reactivation occurred if the powder used had been manufactured from milk preheated at 145° F., whereas reactivation did take place if this preheating temperature was 160° F. or higher. Further, it was found that the powder manufactured from milk preheated to 145° F., after storage at a high humidity for 2 days, followed by 1 week at 37° C., brought about reactivation of the bacterial phosphatase. It is well known that storage of milk powder under such adverse conditions leads to the formation of reducing substances.

Cultured milks

As has already been recorded, no reactivation took place in the presence of pasteurized milk. In view of the results obtained on sterilized milk and milk powder, it was felt that reactivation might be obtained with pasteurized milk provided it contained reducing systems of bacterial origin. For this purpose organisms of the *coli-aerogenes* group were chosen, for organisms of this group have been found to produce a low Eh when grown in pasteurized milk (3). Two cultures were used—*Bact. coli* I and *Bact. cloacae*, for neither of these produce measurable amounts of phosphatase in milk culture.

When pasteurized milk cultures of either of the above two organisms grown for 24 hr. at 26° C. were shocked after admixture with an equal volume of an aged broth culture of 236, reactivation was obtained. By setting up adequate controls it was proved that this reactivation was due to the phosphatase of 236.

In the course of these experiments it was observed that the cultured milks alone, when shocked and subsequently stored, developed a phosphatase reaction and this was shown to be identical with milk phosphatase. On a few occasions previous to this, ordinary commercial pasteurized milk had been shocked but no reactivation had been observed. In view of the positive results obtained on the cultured pasteurized milks, further tests were made on samples of commercial pasteurized milk (H.T.S.T.). These showed that whilst fresh milks of good bacteriological quality gave little or no reactivation, aged samples of poor quality gave better reactivation though at a lower level than similar samples of raw milk.

Although some increase in reactivation has been observed when commercial H.T.S.T. pasteurized milks have been shocked in the absence of oxygen, the fact that the natural reducing system of raw milk is destroyed by commercial pasteurization (3), and that no reactivation of the bacterial phosphatase 236 has been observed in the presence of pasteurized milk even in the absence of oxygen, suggests that the reactivation of milk phosphatase in aged samples of commercial pasteurized milk brought about by shocking is influenced both by utilization of oxygen and the reducing systems by growing organisms. At the same time it is appreciated that the observations of Wilson *et al.* (3) were based upon milk pasteurized by the holder process.

The effect of preheating raw milk on reactivation

As indicated above little or no reactivation was obtained with samples of fresh commercial pasteurized milk after shocking. On the other hand, it had been found that shocked milk in capillaries could be re-shocked immediately, i.e. without any intermediate storage period, with little loss of reactivation provided that the time/temperature combinations used were within the limits already ascertained (1). Since H.T.S.T. pasteurization can be regarded as a form of preheating, further examination of the effect of preheating on subsequent reactivation was made.

Using the capillary technique and using time/temperature combinations known to inactivate milk phosphatase prior to shocking at 135° C./20 sec., it was found that preheating slightly reduced the level of reactivation developed on subsequent storage at 30° C. This fall in reactivation never exceeded 50% of that developed in the non-preheated shocked milk over a wide range of time/temperature conditions, and clearly demonstrates a difference between preheating in the form of commercial H.T.S.T. pasteurization and that conducted in capillaries under equivalent time/temperature conditions.

The effect of re-shocking a reactivated milk

It has already been recorded that no reactivation occurred when a 'reactivated' milk in capillaries was laboratory pasteurized and subsequently stored. (Reactivated milk means shocked milk which, on storage, develops a positive phosphatase reaction.) However, it was found that when a reactivated milk was re-shocked, i.e. when re-shocking took place after several days' storage at 30° C., some reactivation occurred on subsequent storage though this at no time exceeded 50% of that developed in the control once-shocked milk.

Some of the results obtained on the effect of shock treatment and laboratory pasteurization using capillaries are illustrated in Table 6. Shock treatment means 135° C./20 sec. and laboratory pasteurization means 63° C./30 min.

Table 6

Treatment	Reactivation
Shocked once	+ + + +
Shocked twice consecutively	+ + +
Shocked three times consecutively	+ +
Shocked, followed by laboratory pasteurization	±
Shocked, followed by laboratory pasteurization then re-shocked	+ + +
Shocked, stored, re-shocked	+
Shocked, stored, followed by laboratory pasteurization	−

The striking feature of these results is the fact that when laboratory pasteurization is the final heat treatment applied, little or no reactivation occurs on subsequent storage. This agrees with the fact that up to the present no reactivation has been observed in commercial samples of either holder or H.T.S.T. pasteurized milk. On the other hand, when raw milk is shocked, then laboratory pasteurized and then shocked again, considerable reactivation occurs.

Since laboratory pasteurization of a shocked milk had such a marked effect upon reactivation, a more detailed examination has been made of the effect of heating upon shocked milk.

The effect of further heat treatment on shocked milk

The further heat treatment was applied immediately after the shock treatment and its effect upon reactivation determined. In all these experiments, the raw milk used was drawn from bulk supply in rail tanks and was incubated at 37° C. until it gave a disk reading of 2–4 on the 10 min. resazurin test. By this means a high level of reactivation was obtained which enabled a more accurate assessment to be made of the effect of further heat treatment.

Method

The requisite number of capillaries were filled with the incubated raw milk which was cooled to room temperature before use. The capillaries were then shocked at 135° C. for 20 sec. Because of the number involved in any one experiment it was not possible to immerse them all in the glycerine bath at the same time, so successive groups of four were shocked consecutively as rapidly as possible. These unopened capillaries were then mixed and random pairs subjected to reheating for varying times at one particular temperature. It was usually found convenient to examine the effect of reheating to one temperature with one supply of raw milk using a storage time of 2 days at 30° C.

The results which are given in Table 7 are the mean values of at least two experiments.

Table 7. *Relative percentage reactivation after 2 days at 30° C. following reheating*

Reheating temperature (° C.)	Time of reheating						
	0	10 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.
63	100	65	59	57	44	31	22
70	100	84	65	59	48	35	12
80	100	63	59	47	40	33	16
87	100	79	76	66	47	35	16
95	100	97	75	74	53	47	28
105	100	83	74	67	54	37	12
115	100	81	64	62	49	14	Nil
135	100	85	51	22	(6)	Nil	—
145	100	79	49	(9)	Nil	Nil	—
160	100	21	(7)	Nil	—	—	—

Figures in parentheses are approximate since they arise from readings of <0·1.

In view of the considerable effect of reheating shocked milk at 63° C. for even very short times, the effect of reheating at lower temperatures was examined, and it was found that reheating at as low a temperature as 50° C. had a considerable effect, only 5 min. at this temperature resulting in a reactivation loss of 30%.

These results, together with those given in Table 7, are shown in Fig. 1. It is emphasized that these results not only represent mean values of at least two experiments for each

time of reheating, but that many different bulk raw milks were used. A feature of these curves is the presence of peaks at reheating temperatures of 70 and 95° C. The peak at 70° C. is most marked at the shortest reheating time, and has disappeared at the longest reheating time, i.e. 10 min. It is clear that the effect of reheating a shocked milk is a complex one and suggests that two, or even more, effects are operating. The significance of these curves is referred to later under Discussion.

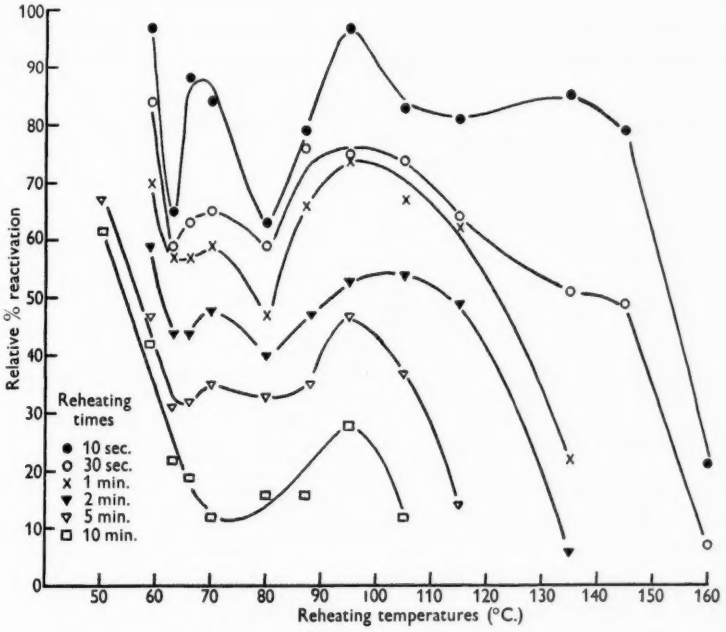

Fig. 1. The effect of reheating a shocked milk upon its subsequent reactivation.

The effect of reheating shocked milks in the absence of oxygen

In view of the known effect of the presence of oxygen on reactivation, the effect of reheating shocked milk in its absence has been examined. Typical results are given in Table 8.

Table 8

	Reheated to	Reactivation	
		In air	In nitrogen
Raw milk, shocked, 130° C./25 sec.	—	0·4+	0·7
Raw milk, shocked, 130° C./25 sec.	63° C./10 min.	0·2−	0·25
Raw milk, shocked, 130° C./25 sec.	63° C./30 min.	Trace	0·1
Raw milk, shocked, 130° C./25 sec.	51° C./60 min.	0·1	0·2−

These results show that the effect of reheating shocked milk in the absence of oxygen is approximately the same as in its presence though at a higher level.

Comparison of the heat-stability of normal alkaline phosphatase of raw milk and the effect of reheating on shocked milk

Owing to the marked effect of reheating shocked milk on subsequent reactivation, an examination has been made of the comparative effect of heat upon shocked milk, i.e. 'reactivable' milk, immediately following the shock treatment, and upon raw milk phosphatase. This comparison was made at a number of temperatures using, on the one hand, raw milk shocked to 135° C./20 sec., and on the other hand, pasteurized milk containing sufficient raw milk to give a reading on the micro phosphatase test approximately the same as that given by the shocked milk control, i.e. no reheating given.

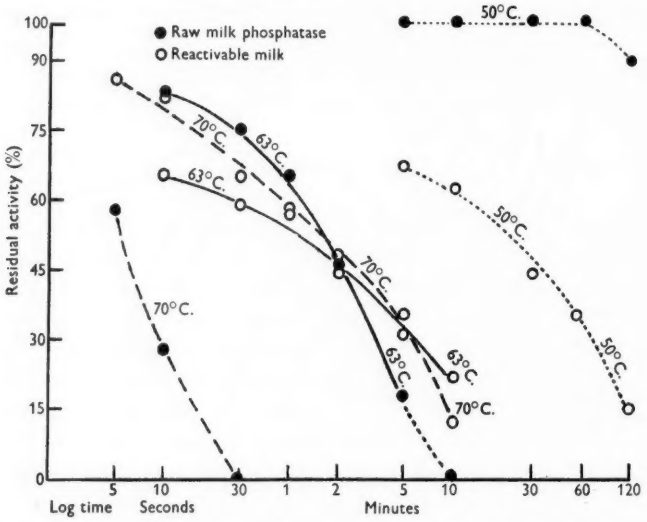

Fig. 2. The heat stability of reactivable milk and of raw milk phosphatase at 50, 63 and 70° C.

The curves in Fig. 2 represent the effect of heat upon the two types of milk at 50, 63 and 70° C., and the results obtained at these and other temperatures are given in Table 9. The comparative tests were made under identical conditions, i.e. by immersion of the capillaries of the two types of milk in the water bath at the same time.

From Table 9 it will be seen that with reheating temperatures below 63° C., the heat stability of the reactivable milk is less than that of a raw milk dilution of the same initial value. Reheating temperatures above 63° C., however, had the reverse effect, i.e. the heat stability of the reactivable milk was greater than that of raw milk phosphatase. Fig. 2 shows these two effects and also the transition stage at 63° C., where the heat stability of the reactivable milk is lower up to 2 min. approx., while greater with longer reheating times.

Whilst studying this effect of reheating on shocked milks, an examination was made on the effect of reheating shocked milks which had been laboratory pasteurized at 63° C. for 30 min. immediately after shocking. This was done since no reactivation had been observed when raw milks were shocked, then laboratory pasteurized and stored for

2 days at 30° C., and therefore the effect of further heating of such a milk might indicate at what reheating temperature reactivation recommenced.

Whilst these experiments showed that reactivation took place when milks shocked and then laboratory pasteurized were reheated to a temperature as low as 80° C., the values obtained were so low (relative % reactivation <20%) at reheating temperatures of less than 100° C., that the experiments were abandoned. Instead a further examination was made of the effect of direct heating of raw milk to temperatures below 100° C.

Table 9

Temperature (° C.)		Percentage residual activity after heating times of										
		0	10 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.	20 min.	30 min.	45 min.	1 hr.	2 hr.
50	A	100	—	—	—	—	98	98	—	98	—	98	90
	B	100	—	—	—	—	67	62	—	44	—	35	15
59	A	100	100	88	88	81	68	52	40	31	15	—	—
	B	100	97	84	70	59	47	42	20	17	0	—	—
63	A	100	83	75	65	46	18	0	—	—	—	—	—
	B	100	65	59	57	44	31	22	—	—	—	—	—
66	A	100	71	53	31	14	0	0	—	—	—	—	—
	B	100	88	63	57	44	32	19	—	—	—	—	—
70	A	100	28	0	—	—	—	—	—	—	—	—	—
	B	100	84	65	59	48	35	12	—	—	—	—	—

A, raw milk phosphatase heated in capillaries. B, reactivable milk, reheated prior to storage.

Direct heating of raw milk to temperatures below 100° C.

It had already been found (1) that reactivation occurred when raw milk was heated to as low a temperature as 84° C. in capillaries. Using bulk raw milk of poor bacteriological quality (10 min., resazurin test reading 2–4) and penicillin at a concentration of 1 unit/ml., further experiments were made in order to determine the lowest temperature of heating which would result in reactivation on subsequent storage.

In all these tests at least three capillaries were used for each time temperature combination, and after storage for 2 days at 30° C., the contents of these were bulked before withdrawing a portion for laboratory pasteurization. The absence of any appreciable yellow colour in the portion tested after laboratory pasteurization was taken as denoting the absence of any phosphatase of bacterial origin. In addition a replicate capillary was tested after 2 days cold storage in order to cover the possibility of residual milk phosphatase being present.

Using these precautions it was found that reactivation occurred at a heating temperature as low as 70° C. These raw milks of poor bacteriological quality always gave excellent reactivation of the order of 0·8 after the usual shock treatment, i.e. 135° C./20 sec. as compared with that obtained (0·2–0·4) when this aspect of reactivation was studied earlier. This explains why no evidence of reactivation was obtained previously (1) at heating temperatures below 84° C.

This finding that raw milk, suitably aged, reactivates after heating in capillaries to as low a temperature as 70° C. (158° F.) naturally led to a reconsideration of the possibility of reactivation occurring in commercial H.T.S.T. pasteurized milk (161° F./15 sec.). At this time a small H.T.S.T. plant (A.P.V.) of 50 gal./hr. capacity became available, and it has been found that certain raw milks will reactivate after pasteurization in this plant at

161° F., though no evidence so far has been obtained of reactivation after heat treatment at this temperature, using ordinary commercial bulk raw milk as received in London. A full account of the work using this small scale plant will be published in a later paper.

Relationship between reactivation and the turbidity test (Aschaffenburg)

It is generally accepted that proteins are an integral part of all enzymes, and it has frequently been suggested that denaturation of the protein plays a part in the inactivation of enzymes by heat. Moreover, it has been suggested (4) that reversion of denatured protein to its native state may play a part in reactivation of enzymes such as trypsin and chymotrypsin.

Experiments were accordingly made to see whether any relationship existed between denaturation of milk proteins and the occurrence of reactivation. For this purpose the turbidity test of Aschaffenburg, which is the official test for sterilized milk in Great Britain (5), was used. A negative result on this test, i.e. absence of turbidity, denotes complete denaturation of the soluble proteins of milk. This test, like the phosphatase test, had to be modified in view of the small volumes of milk available. It was, however, not found possible to obtain a satisfactory test with less than 0·5 ml. milk, and in order to obtain this volume about ten capillaries were used for each test.

The method used was as follows: the contents of the capillaries were emptied into a weighed $3 \times \frac{3}{8}$ in. test-tube, the weight of milk determined, and an equal volume of saturated ammonium sulphate solution added. After vigorous mixing for 1 min., the contents of the tube were filtered through a fluted No. 12 Whatman filter-paper, cut to a diameter of 5 cm. approx., in a small funnel and the filtrate collected in a $2 \times \frac{1}{4}$ in. Durham tube. The tube was then placed in a boiling water-bath for 5 min. before examining its contents for turbidity. If present this was assessed using 'Wellcome' opacity tubes.

This modified test is less sensitive than the official test, being able only to detect 2% raw milk as compared with 0·7% by the official method.

Using this technique it was found that no reactivation took place when the shocked milk gave a negative turbidity test. The maximum times of heating at temperatures over 120° C. for the occurrence of reactivation have been given (1), and it was found that these coincided with those necessary to bring about a negative turbidity test. In this connexion the samples of 'Uperized' milk, which gave rise to this investigation of reactivation, all gave a positive turbidity test.

In addition, it has been shown that the absence of reactivation in reheated shocked milks, as shown in Table 7, also coincides with a negative turbidity test at reheating temperatures of 115° C. and over. Although shocked milk (135° C./20 sec.) when reheated at 63° C. for 30 min. did not reactivate after 2 days' storage at 30° C., slight reactivation did occur after 7 days' storage and the turbidity test was positive on such milk. This positive turbidity test was, however, given by the milk before storage.

Visual examination of the turbidity suggested that some increase did take place as a result of storage at 30° C., but owing to the small volume of filtrate it was not found possible to measure this satisfactorily.

Effect of rate of heating upon reactivation

It has already been stated that much less reactivation was obtained when milk was shocked in small test-tubes (F/D tubes) and mention has also been made that heating

was carried out in a glycerine bath for temperatures in excess of 100° C. and usually in a water-bath for temperatures below 100° C.

In considering the reason for the low reactivation level using small test-tubes, attention was given to the rate of heating since this must be considerably slower in these small test-tubes as compared with capillaries. By determining the residual phosphatase in capillaries of raw milk heated to temperatures over the range 80–100° C. in both a water-bath and a glycerine-bath, it was found that the rate of heating was considerably slower in glycerine. It was thus possible to study the effect of rate of heating by comparison of the reactivation obtained after capillaries had been immersed in a water-bath and a glycerine-bath at the same temperature. The results obtained showed that only a slightly lower reactivation took place after immersion in glycerine, and would therefore seem to confirm our previous statement that the much lower reactivation obtained using small test-tubes is mainly due to oxygen.

The same marked effect on reactivation was found in experiments on the effect of preheating when it was noted that milk preheated in test-tubes, prior to shock treatment in capillaries, showed much lower reactivation than when preheating took place in capillaries. It has already been noted that little or no reactivation occurred when fresh samples of H.T.S.T. pasteurized milk were shocked.

In assessing the effect of rate of heating, however, it is necessary to consider the effect both of holding milk at the bath temperature and also of the relative effect of the bath temperature and lower temperatures on subsequent reactivation. Using capillaries, the effect upon reactivation of holding milk for varying times at two different temperatures is shown in Table 10.

Table 10

Temperature of bath	Immersion time								
	2 sec.	5 sec.	10 sec.	20 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.
95° C. (water)	0·3+	0·35	0·3−	0·3+	0·3	0·3	0·25	0·2−	0·1−
115° C. (glycerine)	0·5+	0·5+	0·5	0·4	0·4	0·3+	0·2+	0·1	Trace

It will be seen from these figures, which were obtained on different milks, that the adverse effect of holding at the bath temperature commences earlier, as expected, at the higher temperature. Since it has been found that for any given immersion time up to 2 min., and for any given milk, the reactivation developed is greater at 95° C. than at any temperature lower than this, a slower rate of heating when capillaries are immersed in a bath at 95° C. will lead to less reactivation for immersion times up to 2 min. approximately. This has been confirmed by direct experiment.

DISCUSSION

In considering the results of the experiments reported in this paper, the following facts seem to have an important bearing upon the development of reactivation: (1) The effect of reheating upon shocked milks. (2) The effect of oxygen and reducing systems. (3) The relation between reactivation and turbidity test results.

(1) The reheating was carried out immediately after the shock treatment and thus represents the effect of this degree of heat upon a milk capable of reactivating when

stored at suitable temperatures such as 30° C. The striking feature of these results, illustrated in Fig. 1, is the existence of peaks in the curves at reheating temperatures of 70 and 95° C., and that the peak in the case of the lower temperature is most marked at the shortest time of reheating and lessens with longer reheating times. At the longest reheating time, i.e. 10 min., this peak at 70° C. is no longer evident.

Examination of the shape of these curves points to the influence of at least two factors, namely a destructive action and the creation of conditions more favourable to reactivation. At reheating temperatures below 63° C. the latter effect is only slight but is evident by the smaller slope of the curves after 5 and 10 min. reheating and confirmed by the curves in Fig. 2 which show the relative increase of the heat resistance of shocked milk as compared with that of raw milk phosphatase, due to the influence of this creative effect. The influence of the creative effect becomes very marked at 70° C., but at this reheating temperature the favourable conditions are very heat labile as shown by the absence of any rise in the curve at all after 10 min. reheating and the fall in the curves as the temperature approaches 80° C. The transient peak at 70° C. is consistent with the fact that when raw milk is shocked in capillaries, reactivation takes place after extremely short times of heating as illustrated in Table 10. As the temperature is raised from 80 to 95° C., conditions more favourable to reactivation again occur but above 95° C. the destructive effect becomes predominant. The sharp fall in the 10 and 30 sec. curves in Fig. 1 above 145° C., suggests the effect of irreversible denaturation of the soluble proteins at these high temperatures.

This creation of conditions more favourable to reactivation was also shown in the reheating experiments carried out on shocked milk which had been laboratory pasteurized immediately after the shock treatment. This laboratory pasteurization results in no reactivation occurring within 2 days at 30° C., but with subsequent reheating temperatures of 80° C. and above, some reactivation did occur.

(2) There is no doubt that the absence of oxygen and the presence of reducing conditions are factors favourable to reactivation. At the same time, it has been found that some raw milks of good bacteriological quality give good reactivation in the presence of air which cannot be increased by replacing the air by nitrogen. At the moment no explanation has been found for this anomaly. The role of reducing conditions was particularly noticeable in our experiments with the bacterial phosphatase (236) which we have been able to reactivate only in the presence of reducing systems, i.e. in the presence of sterilized milk, certain milk powders and milks containing reducing systems of bacterial origin.

A particularly striking example of this was the result of the experiments with milk powder. Reactivation of 236 did occur when the powder used had been manufactured from milk which had been preheated to 160° F. or higher. No reactivation took place in the presence of powder manufactured from milk preheated to 145° F. unless the powder had been previously stored under adverse conditions known to produce reducing substances.

(3) It has been shown that there is a clear relationship between reactivation and the denaturation of the soluble proteins of milk as indicated by Aschaffenburg's turbidity test. That is to say no reactivation has been found to occur when the heating conditions were such as to produce a negative turbidity test. This complete denaturation of the soluble proteins of milk does not explain why no reactivation occurs after 2 days' storage in the case of a milk which is first shocked and then immediately laboratory pasteurized.

Such milks give a positive turbidity test, and while it is true that slight reactivation does occur in such milks after, say, 7 days' storage at 30° C., it may be that the effect of laboratory pasteurization in these cases is to destroy conditions favourable to reactivation.

The reactivation of enzymes has been shown to occur in a number of cases and is now generally accepted. In some of these cases the authors concerned have indicated certain conditions which appear to be favourable to reactivation. Among these is the suggestion that reactivation of enzymes is due to the reversible denaturation of proteins taking place under the influence of SH groups. In considering our findings, we have had in mind the present concept of enzyme structure, namely, that enzymes consist of two components, an apoenzyme and a coenzyme, the former being a protein, and that the closeness of the union of these two components varies. In fact, this latter point has been used as a means of classifying enzymes. Albers (6) has claimed that certain phosphatases are dissociated into these two components by reducing the pH, and Sjöström (7) has attempted to explain the effect of souring of milk on milk phosphatase on this basis.

Bearing this in mind a number of possible explanations of the reactivation of milk phosphatase have been considered. Whilst none of these seem to afford a satisfactory explanation of all the facts presented in this and the preceding paper, we put forward the following tentative explanations:

(*a*) Reactivation occurs as the result of the reversion of the denaturation of the apoenzyme taking place under the influence of reducing conditions. This presupposes that the inactivation of an enzyme is due, at least in part, to the denaturation of its protein moiety. The stability of the alkaline phosphatase of raw milk to heat in the absence of moisture, which we have found to be very marked, lends support to this view. The process of reversion of denaturation would be expected to be a function of time and probably of temperature, which would conform with our findings on the effect of storage time and temperature. The absence of any evidence up to the present of reactivation in either laboratory pasteurized or commercial pasteurized milk (holder or H.T.S.T.) can be explained as being due to the absence of the formation of reducing conditions as a result of these particular forms of heat treatment. This is consistent with the fact that the production of reducing conditions or systems in raw milk by heat does not become evident, at any rate as far as chemical tests are concerned, until the heating temperature rises above 70° C. Within the wide range of time/temperature combination permitting of reactivation, reducing systems are undoubtedly established in the milk. The absence of reactivation when the heat treatment has been severe enough to bring about a negative turbidity test is due to the irreversible denaturation of the apoenzyme.

The effect of reheating shocked milks can be explained as being due to the influence of reheating on the reducing conditions or systems in these milks, together with a possible direct effect on the apoenzyme.

(*b*) Inactivation of milk phosphatase by heat takes place as a result of inactivation of the coenzyme, reactivation of the phosphatase being due to the formation of a new coenzyme brought about by the heat treatment given. The association of the new coenzyme with the apoenzyme is a time reaction and explains the effect of storage time and temperature on reactivation. The absence of reactivation after heat treatment sufficient to give a negative turbidity test is again due to the complete denaturation of the apoenzyme.

The production of this new coenzyme and the effect of heat upon it is reflected in the

reheating curves, but it is suggested that it is closely connected with the production of reducing systems. In this explanation the apoenzyme is assumed to be relatively heat stable as compared with the coenzyme, whereas in (*a*) the apoenzyme is assumed to be heat labile and the coenzyme heat stable.

If it is assumed that laboratory pasteurization of raw milk results in irreversible inactivation of the natural coenzyme, then it would be logical to explain the effect of laboratory pasteurization on shocked milk as being due to irreversible inactivation of the new coenzyme.

This explanation implies that the characteristics of milk phosphatase so far examined, i.e. pH optimum, heat resistance and hydrolytic power with various buffer substrates, reside in the apoenzyme. Our findings on the conditions which affect the reactivation of the bacterial phosphatase 236 support this explanation.

(*c*) Inactivation of milk phosphatase by heat is due to the destruction of some essential link which enables apoenzyme and coenzyme to function as an active unit. No reactivation occurs as a result of laboratory or commercial pasteurization because this link is destroyed, but more severe heat treatment results in the formation of a new link under the influence of reducing conditions. Again, as in the other two hypotheses, the union of apoenzyme and coenzyme by this new link is assumed to be a time/temperature reaction. The effect of reheating shocked milk can be explained as under (*b*).

Each of these possible explanations is consistent with the fact that reactivation has only been found to occur to the extent of 5% (expressed as raw milk phosphatase) and each of them leaves unexplained certain facts.

In conclusion, it is clear that a final and satisfactory explanation of reactivation must await further evidence.

SUMMARY

1. Using the capillary technique previously described, examination has been made of various factors in relation to the reactivation of raw milk phosphatase. Of these, ageing of the raw milk was found to affect considerably the level of reactivation, this increasing as the bacteriological quality of the milk deteriorated.

2. It has been found that absence of air and the presence of reducing conditions in the raw milk increases the reactivation obtained.

3. The influence of reducing conditions on the reactivation of a bacterial phosphatase has been demonstrated.

4. The effect of reheating shocked milks prior to storage has been examined over a wide range of time/temperature combinations. In all cases reactivation was diminished by this additional heat treatment, but was not directly proportional to the temperature, being least at 95° C.

5. By using the Aschaffenburg turbidity test it has been found that reactivation does not take place when milk is heated sufficiently to denature the soluble proteins completely.

6. The role played by some of the factors found to affect reactivation is discussed.

7. Experimental work on reactivation has been carried out using a small H.T.S.T plant (A.P.V.). Whilst certain milks have developed a positive phosphatase test after pasteurization at 161° F., no reactivation has been obtained using commercial bulk raw milk as received in London. An account of this work will be published in a further paper.

In conclusion the authors wish to express their thanks to Dr R. J. MacWalter for his continuing interest in this work.

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518. THE PROBLEM OF FISHINESS IN BUTTER

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Fishiness is now rarely found in freshly made butter because of improvements in the method of manufacture. Indeed this defect develops only occasionally in butter after lengthy storage and its cause is still obscure.

There are two main theories as to the cause of fishiness—(*a*) biological, and (*b*) chemical.†

(*a*) *Biological theories*

Attempts to reproduce fishiness by bacterial action have, in general, proved unsuccessful. The main objections to this theory are (i) pasteurization does not completely eliminate fishiness, (ii) inoculation experiments with micro-organisms suspected of causing the defect are usually unsuccessful, and (iii) fishiness is favoured by high salt content and develops at low temperatures, and such conditions are not conducive to microbial activity.

(*b*) *Chemical theories*

Rogers (17) was one of the first to advance a chemical theory and study the mechanism of fishiness development, though he did not agree with Sommerfeld (21) that fishiness was due to trimethylamine. Supplee (23) supported Sommerfeld's theory, although unable to isolate this amine from all fishy samples analysed, and Cusick (3) agreed with him that the amine was derived from the phospholipid lecithin. Both Supplee and Cusick believed the defect to be of bacterial origin, but Sommer & Smit (20) showed that the reaction could proceed by purely chemical means. Fishy butters analysed by these authors contained 23–36 p.p.m. of trimethylamine, corresponding to about one-third to one-half of that potentially available from the lecithin present. Sommer & Smit also found that fishy flavour developed more readily in butters to which lecithin had been added. Barnicoat & Palmer (2) considered that kephalin, which contains the base ethanolamine, rather than lecithin was the phospholipid concerned.

Ritter (16) and Davies & Mattick (6) also supported the trimethylamine theory. The latter authors postulated the oxidative degradation of the choline of lecithin by peroxides formed by oxidation of unsaturated fatty acids, but Davies & Gill (5) later abandoned this theory as the trimethylamine found in their experiments fell far short of expectations. They suggested that low molecular weight nitrogenous compounds (derived possibly from proteins) enter into combination (of unknown nature) with fats giving rise to fishy flavoured substances. Other workers, e.g. Mohr & Arbes (14), failed to isolate trimethylamine from fishy butters, while Storgards & Hietaranta (22) concluded that decomposition products of the more highly unsaturated fats are responsible.

* Holder of Catherine Baxter Scholarship during tenure of which results included in this paper were obtained (in partial fulfilment of the requirements of the degree of M.Agr.Sc.).

† Investigations prior to 1923 have been thoroughly reviewed by Sommer & Smit (20).

Although none of the suggested chemical mechanisms have been shown conclusively to be associated with fishiness it is generally agreed that certain factors influence its development in butter, viz.:

(i) *Metallic oxidation catalysts.* Various workers (5,6,11,16,17,20) have emphasized the importance of traces of copper (and to a less extent, iron) in promoting this defect.

(ii) *Acidity.* Butters of low pH, made from high-acid creams, have inferior keeping qualities and tend to develop fishiness, especially when salted (10,17,20,24). Lowering the churning acidity was an important factor in eliminating fishiness in New Zealand export butters in the early days of the industry (15).

(iii) *Pasteurization.* A number of workers, including those first reporting on the problem (3,11,15,16,17,20,23), have emphasized the importance of pasteurization in controlling fishiness.

(iv) *Feed.* Many feedstuffs, particularly oily substances, have been blamed for causing fishy flavours in dairy products. Davies (4) found that sugar-beet tops contained betaine which appeared as trimethylamine oxide in milk and gave fishiness on bacterial reduction to trimethylamine.

(v) *Inorganic substances.* It is generally agreed that a high salt concentration encourages fishiness, though whether it arises from the peptizing action of the brine on the proteins, or lecithoproteins, bringing them into closer association with the oxidizable fat, or from other causes, is unknown (17,20). Borates, which retard microbial growth, were found to promote fishiness in butters (1). The trivalent borate ion would have a more powerful peptizing action than chloride.

(vi) *Temperature level of storage.* The development of the taint is retarded but not eliminated by storage at low temperature.

(vii) *Overworking of butter.* Overworking butter is considered to predispose it to fishiness, either because the action of overworking makes the phospholipid or protein more soluble in the brine, or because of the incorporation of extra air which promotes oxidation (17).

Many of the earlier theories are difficult to interpret because of the interference of uncontrolled factors, especially in biological experiments. Results, on the whole, support the chemical theory.

In this paper experiments are described in which deliberate attempts were made to produce fishiness by manufacturing butters under conditions reputed to cause it. Various factors reported as promoting fishiness were studied individually and in combination, and the resulting butters were examined at intervals critically for flavour and for chemical changes as indicated by degree of fat oxidation and trimethylamine content.

EXPERIMENTAL

(1) *Methods*

(a) *Buttermaking*

Butters were made from mixed sweet creams containing approximately 40% fat. The cream was held before and after pasteurizing in stainless steel or glass vessels. Pasteurization was carried out at approximately 200° F. in a Vacreator or flash pasteurizer. After cooling the cream to 40° F., additions were made of hydrochloric acid or sodium hydroxide

solutions to adjust pH,* also of traces of copper when required and the creams were then held overnight at about 40° F. Churnings were made either in a wooden factory churn or in a small, well-tinned metal churn. When using the small metal churn, the butter was worked on a wooden table worker, and salt was added to the cream just before churning, and to the chilled wash water at the same concentration as in the butter 'serum'. Particular care was taken to prevent microbial contamination by using copious amounts of boiling water for cleansing.

Over fifty experimental churnings were made. The butters were divided into several portions and packed in parchment-lined cans which were well sealed.

(*b*) *Storage*

The tinned butters were stored at 60–65° F. for periods varying from about 3 to 20 weeks.

(*c*) *Grading*

Butters were graded after approximately 1, 2 and 5 months' storage by experienced butter graders.

(*d*) *Chemical methods*

pH *determination*—by quinhydrone electrode.

Proximate analysis of butter—by factory routine methods.

Fat oxidation tests—Lea's modified peroxide test (12) and Schibsted's fat aldehyde test (19).

Trimethylamine—the method of Dyer (7, 8) was adapted for this study as follows:

Extraction of amines of butter. To 30 g. of butter in a 100 ml. flask were added 1 ml. 20% trichloroacetic acid and N/500-HCl to bring the aqueous layer to 15 ml. After shaking with 30 ml. chloroform, the mixture was maintained for 2 hr. at 38° C. and then centrifuged.

Estimation of amine as picrate. 4 ml. of the supernatant aqueous layer were shaken well in a glass-stoppered vessel with 10 ml. toluene, 1 ml. formaldehyde and 3 ml. potassium carbonate solution. 7–8 ml. were transferred to a tube containing recently dried sodium sulphate (closed with a cellophane-covered cork), and allowed to stand for 15 min. 5 ml. of the toluene extract were mixed well with 5 ml. 0·005% toluene-picrate reagent and the colour intensity was measured at 412 mμ in a Beckman spectrophotometer (model DU).

The method gave good recoveries (±5% error) when trimethylamine ranging from 2·5 to 20 p.p.m. was added to butter. Non-volatile amines were present only in negligible amounts.

(2) *Buttermaking trials*

Experimental churnings were planned to ascertain the effects of the following factors (singly and in combination) on the development of fishiness:

(i) Acidity—pH range 5·5–7·2.
(ii) Salt content of butter—nil to 5·4%.
(iii) Milk enzymes—by using raw cream.
(iv) Chemical products of soured (and repasteurized) cream.

* Since lactates have antioxidant properties (13).

(v) Traces of oxidation catalyst (0·5 to 2·5 p.p.m. copper).

(vi) Borates.

The programme of experimental churnings is shown in Table 1.

Table 1. *Experimental butter churnings*

No. of exp.	Factors studied	Series	Cream: Treatment or additions	Cream: Heat treatment	Cream: pH	Salt content of butter
I	(i) Heat treatment	*a*	Acidified with HCl	Raw	6·8, 6·3, 5·5	1·5%
	(ii) pH	*b*	Acidified with HCl	Pasteurized	6·8, 6·3, 5·5	1·5%
		c	Soured, neutralized, pasteurized, acidified	Pasteurized	6·8, 6·3, 5·5	1·5%
II	Salt content (pasteurized cream)	*a*	—	Vacreated	7·2	Nil
		b	—	Vacreated	7·2	0·9
		c	—	Vacreated	7·2	4·5
		d	—	Vacreated	7·2	5·4
III	(i) Salt content	*a*	—	Vacreated	6·7, 5·5	Nil
	(ii) pH (pasteurized cream)	*b*	—	Vacreated	6·7, 5·5	1·4
		c	—	Vacreated	6·7, 5·5	4·0
IV	(i) Salt content	*a*	—	Raw	6·8, 5·5	Nil
	(ii) pH (raw cream)	*b*	—	Raw	6·8, 5·5	1·7
		c	—	Raw	6·8, 5·5	3·4
V	(i) Salt content	*a*	To one series nil; to another series 0·5 p.p.m. copper and to the third series 2·5 p.p.m. copper (fat basis)	Vacreated	6·8, 5·5	Nil
	(ii) pH	*b*		Vacreated	6·8, 5·5	1·5
	(iii) Traces of copper (pasteurized cream)	*c* etc. to *i*		Vacreated	6·8, 5.5	3·2
VI	Borate content in salted butters (pasteurized cream)	*a*	No borate	Vacreated	7·2	1·3
		b	0·65% borate buffer mixture	Vacreated	7·2	1·3
		c	1·4% borate buffer mixture	Vacreated	7·2	1·3

Except for the experimental treatments imposed, the butters were of normal make, and contained about 15% moisture and 1·1% curd.

Extensive series of results of analyses and gradings of these butters carried out after different periods of storage are summarized as follows:

(i) Unsalted butters usually developed a cheesy flavour.

(ii) 'Tallowiness' was promoted by high salt content, traces of added copper, and low pH.

(iii) Metallic flavours were not restricted to butters containing added copper and indeed were more common in butters to which no metal had been added.

(iv) 'Storage' flavour occurred most frequently in butters manufactured according to normal New Zealand practice, i.e. in pasteurized cream butters, of pH 6·8 and containing about 1·5% salt.

(v) No fishy flavour whatever was detected, and none of the samples contained more than 3 p.p.m. trimethylamine.

(vi) Fat peroxide- and fat aldehyde-values varied considerably, though the peroxide values, were, on the whole, of low magnitude (below 0·5). Fat oxidation values were highest for butters containing added copper, or high amounts of salt, or of low pH. They tended to decrease with length of storage of butter, probably because of their degradation to further products of fat oxidation.

DISCUSSION

In a series of experimental butters manufactured deliberately under conditions reputed to produce fishy flavours, no fishiness whatever was detected even after prolonged storage of the products. This may appear surprising, particularly as other flavour-defects such as cheesey, metallic, tallowy and 'storage' flavours were common. The butters given the most drastic experimental treatments (i.e. containing added copper or excess salt, or of low pH) were, in fact, inedible and bleached at the end of the experiment. As was expected, butters made under less drastic conditions, or held in storage for short periods, were less strongly flavoured, but in no case was there any suggestion of a fishy taint.

It might be contended that insufficient phospholipid was present to permit the taint to be perceived, for Sommer & Smit (20) found that fishy butters contained 23–36 p.p.m. of trimethylamine. Haylock (9) found, however, that New Zealand factory butters contain, on the average, about 0·1 % phospholipids. One half of this is lecithin, which is equivalent to about 40 p.p.m. available trimethylamine. In these experiments, the trimethylamine estimated by the modified Dyers method (7, 8) never exceeded 3 p.p.m. It is possible, too, that trimethylamine might have undergone further oxidation to trimethylamine oxide, an odourless compound, but none was detected in alcoholic extracts of a selection of the final samples examined by Ronald & Jakobsen's method (18).

There are apparently two types of chemical change responsible for substances perceived as fishiness. These substances are (*a*) ammoniacal products, as found in decomposing non-oily fish and lean meat, probably volatile amines, and (*b*) degradation products from oxidation of the more highly saturated fats which impart a characteristic 'fish oil' flavour (22).

In our experience, the few fishy butters shown to us, or produced experimentally (1) have certainly been of the oily type. It was not, therefore, anticipated that fishiness associated with volatile amines would appear in these experimental butters, but it certainly was expected that some of the butters prepared under conditions promoting fat oxidation (low pH, high salt and copper contents) would develop fishy flavours, particularly on keeping. This, however, was not the case, and even butters containing a borate-buffer mixture made under similar conditions to those of an earlier experiment (1) and, indeed, in the same factory, failed to develop the oily type of fishy flavour previously noted. The presence of milk enzymes (in raw cream butters), or of the chemical products of natural souring (including lactates) were also of no importance.

The theory that fishiness in butter is caused by bacterial action is not now generally held because of difficulties in producing the fault under conditions which do not exclude the possibility that chemical action is responsible. The results described in this paper show that even when chemical factors reputed to cause fishiness were deliberately applied to a series of experimental churnings of butters, they proved ineffective in promoting this defect. Clearly there are still unknown factors concerned in the chemical or biochemical changes responsible for the development of fishy flavour in dairy products.

SUMMARY

Chemical factors reputed to promote fishiness in butter were studied. Attempts were made to reproduce fishiness in experimental churnings of butter by deliberately applying factors reputed to cause the defect, viz. low pH, high salt content, lack of pasteurization, traces of copper, borates—singly and in combination.

After storage at 60–65° F. for 1–5 months, the butters developed various off-flavours—cheesey, tallowy, metallic, storage—but in no case was fishiness observed. At no stage were significant amounts of trimethylamine detected, nor of trimethylamine oxide, though the butterfats were considerably oxidized.

The nature of fishiness is discussed. It is clear that chemical theories cannot adequately account for the development of this defect in butter.

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519. THE COOLING EFFICIENCIES AND WATER PRESSURES OF SOME SURFACE MILK COOLERS FOR FARMS

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(With 6 Figures)

INTRODUCTION

The surface cooler is the only type of farm milk cooler widely used in New Zealand. The construction of these coolers is such that milk flows from a top tray containing a series of small holes or slots, down the outside of the metal cooling surface to a collecting tray. The cooling medium is inside. In most farm milk coolers the cooling surface is composed of a vertical bank of horizontal tubes, but some coolers have a corrugated continuous surface. Fig. 1 shows typical arrangements.

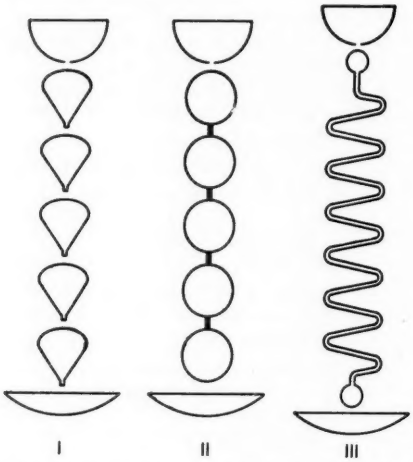

Fig. 1. The three basic types of surface coolers which were tested. I, with gaps between the tubes (tubes illustrated are pear-shaped); II, spaces between the tubes joined; III, corrugated plate design.

Surface milk coolers are known sometimes as Baudelot coolers, capillary coolers and sometimes as milk aerators. In other applications (e.g. ammonia condensers in refrigerating systems) they are designated as trombone coolers, trickle coolers, drip-feed coolers, serpentine coolers, cascade coolers, horizontal-film coolers and S-type coolers.

The majority of farm milk coolers use cold water as the cooling fluid, this investigation being concerned only with this type. Other types use cold water to accomplish part of the cooling and either direct refrigeration or chilled water to complete the cooling.

The quality of milk as delivered to the treating or processing station depends considerably on the temperature history of the milk from the time it leaves the cow, the critical point being the final temperature to which the milk is cooled on the farm.

During summer months much of the milk leaving the farms is not adequately cooled, and in order to help to rectify this position an investigation of the efficiency of cooling equipment was undertaken. It was decided to commence the investigation by an examination of the basic cooling efficiencies of farm milk coolers.

PART I. THE COOLING EFFICIENCIES OF FARM MILK COOLERS

An efficient cooler is one in which the temperature of the milk leaving the cooler is close to the temperature of the cooling water. To determine the efficiencies of several types of milk coolers, testing apparatus was constructed.

Testing procedure

New coolers, representative of those in use, were supplied by manufacturers and retailers, and were tested by means of the equipment shown diagrammatically in Fig. 2.

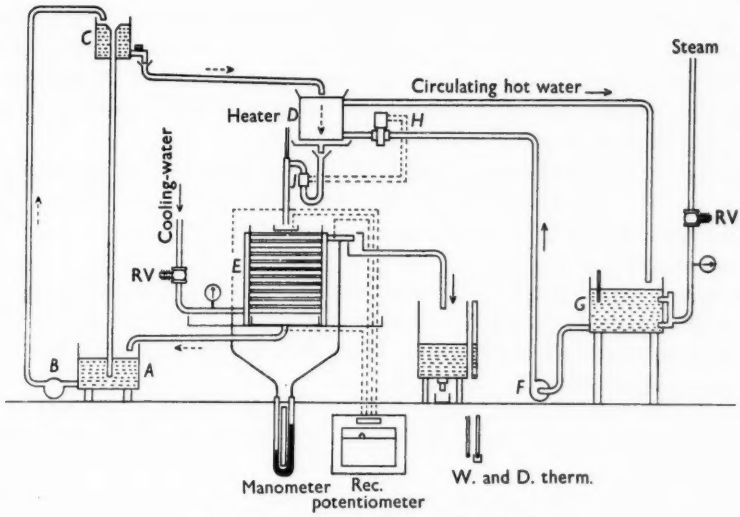

Fig. 2. Arrangement of testing equipment

The milk (or other liquid) was placed in the reservoir *A* and pumped by means of a positive displacement vane pump *B* to a constant level tank *C*. By means of a tap on *C* the flow was adjusted to any rate suitable for the cooler under test. From *C* the liquid continued over a heater *D* (where its temperature was increased to 95° F.) to the cooler under test *E*, and then was returned to the original reservoir *A*.

Cooling-water pumped from a well-bore was supplied through a reducing valve. For the heater *D*, heat was supplied by hot water circulated by a centrifugal pump *F* from a hot-water tank *G*, thermostatically controlled. The steam required to heat the hot water was supplied through a reducing valve and injected into the water through the combined water-steam mixer and thermostat. A motorized valve *H* in the hot water line connected to a temperature controller *J* in the milk flow line provided proportional control to keep the temperature of the milk entering the cooler at 95° F.

The loss of pressure of the cooling-water between inlet and outlet was measured by a mercury manometer. Milk and cooling-water rates of flow were measured by taking the time required to fill a suitable container. Temperatures at various places in the cooler were measured by an electronic, 6-point, dot-printing, self-balancing, recording potentiometer connected to copper-constantan thermocouples. The wet-bulb and dry-bulb air temperatures were measured by a whirling hygrometer.

Warm water at 95° F. was used in the tests instead of milk. The advantages of this procedure are: (1) water is a more convenient fluid to use; (2) a more accurate comparison of the performances of several designs or variations of one design is possible; (3) variations and anomalies due to the complex structure of milk are absent.

A surface cooler can cool warm water to a greater degree than it can cool an equal flow of milk at the same temperature, but any cooler which can cool the outside water to within 3° F. (as specified above) of the inlet cooling water temperature is efficient, and may be considered efficient for all practical purposes when milk is being cooled.

In any one test run, the rate of flow (lb./hr.) of warm water over the outside surface of the cooler was kept constant while the cooling-water flow rate was varied from zero to a value higher than would be encountered in practice. Although most of the results presented here are for one outside water flow only, each cooler was tested at other flow rates also. From curves drawn through plotted points the cooling efficiency at any ratio of cooling-water to warm water could be evaluated.

It should be noted that the results were obtained with clean coolers. Where, in practice, a deposit forms on the metal wall (cooling-water side) the cooling efficiency becomes less. This aspect of the problem will be investigated at a later date.

The coolers tested were:

(1) Four coolers from one manufacturer, all have $1\frac{1}{4} \times 1\frac{1}{4}$ in. pear-shaped tubes. Three of these coolers had nine tubes but one had tubes twice as long as either of the other two. The remaining cooler had eleven tubes. There were non-uniform gaps between tubes measuring approximately $\frac{1}{8}$ in.

(2) One cooler having $1\frac{3}{4} \times 1\frac{3}{4}$ in. pear-shaped tubing similar to the tubing used in dairy factory surface coolers for milk. There were gaps between the tubes measuring approximately $\frac{1}{4}$ in.

(3) One corrugated plate cooler having a constant width narrow gap between the plates for the passage of the cooling-water. There were fourteen 'flats' each 2 in. in length.

(4) One cooler having circular tubes of 1 in. outside diameter with a small lip on the bottom of each tube. The sixteen tubes were divided into a top section of eight tubes and a similar bottom section. In each of the sections the bottom of one tube was joined to the top of the one lower down. Thus the only gap in the surface was that separating the two sections. This cooler is normally used with the top section as a water cooler and the bottom section with a refrigerant. In these tests the sections were connected in series and both used with cooling-water. A separate tray to give higher flows was constructed.

Five of the above coolers each had a surface area of about 8 sq. ft. (ranging from $7\frac{1}{2}$ to $8\frac{1}{2}$ sq. ft.) and each of the two small ones, in class 1, had a surface area of about one-half this amount. The two small coolers were made for a series of special tests, the results of which will be reported later.

Details of the coolers used for the test are given in Tables 1 and 2.

Table 1. *Types of farm milk coolers tested*

Code letter	Country of manufacture	Type of design	Nominal milk cooling capacity (lb./hr.)	Shape of tubes	Type of metal surface
A	N.Z.	Tubular	500	Pear	Tinned copper
B	N.Z.	Tubular	500	Pear	Tinned copper
C	N.Z.	Tubular	1000	Pear	Tinned copper
D	N.Z.	Tubular	1000	Pear	Tinned copper
E	N.Z.	Tubular	1000	Pear	Tinned copper
F	U.K.	Corrugated plate	1250	—	Tinned copper
G	U.S.A.	Two-section tubular with closed gaps in each section	—	Circular with bottom lip	Tinned copper

Table 2. *Principal dimensions of farm milk coolers tested*

Code letter	Outside dimensions of tubes (in.)	Number of tubes	Internal cross-section area (sq. ft.)	Length of tubes for cooling surface (ft.)	Vertical height of cooling surface (ft.)	Surface area (sq. ft.)
A	$1\frac{1}{4} \times 1\frac{1}{4}$	9	0·0066	1·31	1·00	3·45
B	$1\frac{1}{4} \times 1\frac{1}{4}$	9	0·0066	1·31	1·00	3·45
C	$1\frac{1}{4} \times 1\frac{1}{4}$	9	0·0066	2·83	1·00	7·45
D	$1\frac{1}{4} \times 1\frac{1}{4}$	11	0·0066	2·35	1·22	7·55
E	$1\frac{3}{4} \times 1\frac{3}{4}$	5	0·0105	3·66	0·82	8·00
F	—	—	—	1·45	1·11	8·00
G	1	8+8	0·0043	1·92	1·55	4·25+4·25

The figures for nominal milk-cooling capacities were supplied by the manufacturers of the coolers.

Results of tests

One group of results given in Table 3 shows the difference in temperature between the leaving water (entering temperature of 95° F.) and the incoming cooling-water (entering temperature of 65° F.) when the ratios of cooling-water to warm water are (*a*) 3:1, (*b*) 2:1.

The final column gives the ratio of cooling-water to warm water in order to reduce the above-mentioned temperature difference at the bottom of the cooling surface to 3° F. Results are corrected for a cooling-water temperature of 65° F. Air temperatures were 65–75° F.

The effectiveness of each square foot of cooling surface in transferring heat from the warm fluid to the cooling fluid is of fundamental importance in these or any other heat exchangers.

Table 4 shows the range of heat transfer effectiveness (of each square foot of a cooler) for the coolers tested, corrected for a cooling-water temperature of 65° F. Air temperatures were 65–75° F.

Fig. 3 shows the allowable rate of warm water flow (in lb./hr.) for each square foot of cooler surface, for values of the cooling-water rate of flow. The results are given for each of the four full-size coolers *C*, *E*, *F* and *G*.

Discussion of results given in Tables 3 *and* 4 *and Fig.* 3

Table 3

For each cooler the rate of flow of warm water was adjusted until the water-level reached a constant height at the usual working level (in the top tray) for coolers *A* to *D*. For the remaining coolers the flow was adjusted to approximately 1000 lb./hr. For cooler *C*

Table 3. *Results of tests: differential cooling temperatures at various water ratios*

Code letter (1)	Nominal milk cooling capacity (lb./hr.) (2)	Warm water (95° F.) flow over outside of cooler (lb./hr.) (3)	Temperature difference between cooled water and cooling water entering (° F.) 3:1 (4)	2:1 (5)	Required water ratio to cool to 3° F. difference (6)
A	450	321	3·9	6·8	3·8
B	450	320	4·8	7·5	4·4
C	1000	968	4·2	6·4	3·8
D	1000	716	2·4	4·5	2·6
E	1000	1007	7·0	9·8	6·0
F	1250	1018	3·8	5·4	4·0
G	—	990	3·3	5·3	3·1

Table 4. *Effectiveness of heat transfer surfaces*

Code letter (1)	Nominal milk capacity per sq. ft. of cooling surface (lb./hr. per ft.²) (2)	Allowable warm water flow at 3:1 ratio and 3° F. temperature difference at bottom of cooler (3)	Flow capacity (from column 3) per sq. ft. of cooling surface (lb./hr. per ft.²) (4)
A	131	200	58·0
B	131	140	40·6
C	134	565	76
D	132	810	107·0
E	125	270	33·8
F	156	850	106·0
G	—	970	114

the original flow was 720 lb./hr., but the holes in the top tray were drilled to a larger size to allow a flow of 1000 lb./hr.

Coolers *A–D*, all from one manufacturer, had maximum rates of flow only of 72 % of the nominal capacities specified by the manufacturer.

Based on the rates of flow indicated in column 3, the figures in column 4, show that only cooler *D* gave adequate cooling of 3° F. (or less) between the leaving cooled water and the incoming cooling-water. Also, cooler *A* was able to produce a greater degree of cooling than cooler *B*, although both coolers were manufactured to the same plan and specification. The cause of the abnormal behaviour of coolers *A* and *D* was found to be an increase in turbulence at the tube entrances due to a defect in the cast brass headers. Abnormally high water pressures were required for these two coolers.

Of the remaining standard size coolers (approximately 8 sq. ft. of surface and 1000 lb./hr. capacity) the (8 + 8) tube × 23 in. wide cooler *G* was the most efficient, but it had slightly more surface area than the others. The worst cooler was *E* which used $1\frac{3}{4} \times 1\frac{3}{4}$ in. pear-shaped tubing. As shown in column 6, cooler *E* would require nearly 100 % more cooling water than cooler *G* to produce the desirable cooling efficiency.

Table 4

Although Table 3 shows the cooling temperatures to be expected at a warm water flow of 1000 lb./hr., it is instructive to present the data in the form of the allowable capacity to achieve adequate cooling, as shown in Table 4. Column 4 shows clearly the allowable flow for each square foot of cooling surface for each of the coolers tested. Thus cooler *F* could cool a quantity of milk in about one-third the time taken by cooler *E*, both

coolers using 3 gal. of water to 1 gal. of milk. Although in Table 3 the difference in cooling efficiency between coolers *C* and *F* appears small, Table 4 shows that the allowable flow of cooler *F* is 45% greater than cooler *C*.

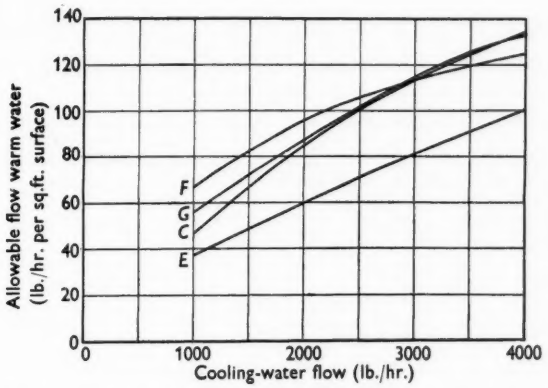

Fig. 3. Cooling capacity in lb./hr. per sq. ft. of cooling surface to achieve 90% efficiency. *C*, 1·25 in. tubular; *E*, 1·75 in. tubular; *F*, corrugated plate; *G*, 1·0 in. tubular no gap.

Fig. 3

This figure shows clearly the relative values of the four main types of design in current use.

The corrugated plate cooler *F* has a greater allowable flow than the other coolers when the rate of cooling-water flow is less than 2900 lb./hr. For a cooling-water flow of 1000 lb./hr. the required surface area of a cooler of a design represented by *F* would be considerably smaller than other designs for the same milk capacity.

The allowable flows per square foot for coolers *G* and *C* are equal at 2800 lb./hr. and the allowable flows for cooler *E* are low over the whole normal range of cooling-water flows.

Derived results

Cooler efficiency

Although it is often convenient to consider a milk cooler efficient when it can cool milk or water to within a few degrees Fahrenheit of the cooling-water inlet temperature a precise definition is, Efficiency is equal to

$$\frac{\text{temperature difference between inlet and outlet of warm fluid}}{\text{temperature difference between warm fluid inlet and cooling water inlet}'}$$

or, in other words, the actual temperature cooled divided by the maximum possible cooling. For instance, a cooler may be supplied with water at 95° F. which has to be cooled by water at 65° F. The lowest temperature the cooled water can reach is of course 65° F. or a temperature drop of 95 – 65 or 30° F. In practice a temperature greater than 65° F. would be achieved—say 68° F. The actual cooling is thus 95 – 68 or 27° F. The efficiency of the cooler is then $\frac{27}{30}$ or 0·9 and generally expressed as 90%.

The temperature difference between the leaving cooled fluid and the inlet cooling-

water (3° F. in the above example) is called the approach temperature. The approach temperature can always be calculated from the percentage efficiency because

$$\text{Approach temperature} = \frac{(100 - \%\ \text{efficiency})\ (\text{maximum cooling})}{100}.$$

Thus in the example above the efficiency is 90%; the maximum degrees it is possible to cool the warm fluid is $95 - 65 = 30$ hence the approach temperature is $\frac{10 \times 30}{100}$ or 3° F.

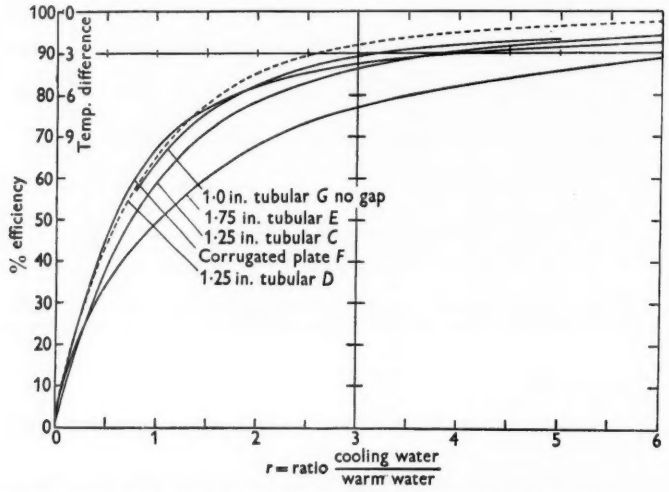
Temp. difference
% efficiency
1·0 in. tubular G no gap
1·75 in. tubular E
1·25 in. tubular C
Corrugated plate F
1·25 in. tubular D
r = ratio cooling water / warm water

Fig. 4. Cooling efficiencies at 1000 lb./hr. for a range of cooling-water ratios.

Fig. 4 shows the percentage efficiency of five coolers each tested with warm water at 95° F. and a flow of approximately 1000 lb./hr., with the exception of cooler *D* (shown dotted) in which the flow was 720 lb./hr. The optimum point at the intersection of the 3:1 cooling-water line with the 90% efficiency line is shown on the graph. A scale of approach temperature is shown also, for a case of a 30° F. maximum cooling range.

The graph shows clearly that as the rate of cooling-water flow decreases the efficiency of cooler *F* does not decrease at as fast a rate as the other coolers. At 1000 lb./hr. of warm water no cooler reached the optimum operating point, although cooler *G* was quite close.

The efficiency of any cooler can be increased to any desired value by decreasing the flow of warm water. Fig. 5 shows the effect of a change in warm water flow on the efficiency of a corrugated plate cooler (*F*) and a tubular cooler made of $1\frac{3}{4}$ in. pear-shaped tubing (*E*). Fig. 5 shows that the efficiency of cooler *F* is affected to a greater extent by outside flow conditions than is cooler *E*. A 100% increase in the rate of flow of warm water causes a smaller fall in efficiency for cooler *E* than for cooler *F*.

Heat lost to atmosphere

The heat lost to the atmosphere was computed by subtracting the heat gained by the cooling-water from the heat lost by the warm water on the outside of the cooler. In all cases the cooling effect of the atmosphere was small. Typical figures were 1·2% more heat removed at 3:1 cooling-water and 8% at 1:1.

CONCLUSIONS

(1) None of the coolers gave the required efficiency. In general, the efficiency increased rapidly as the ratio of cooling-water flow increased to about 3:1, but any increase beyond this point made a smaller increase in efficiency.

(2) The efficiencies of the tubular coolers decreased considerably as the diameter (or cross-sectional area) of the tubes was made larger, which indicates that in tubular coolers the transfer of heat from the warm to the cold water is limited mainly by the difficulty of transferring heat from the metal wall of the tube to the cooling-water.

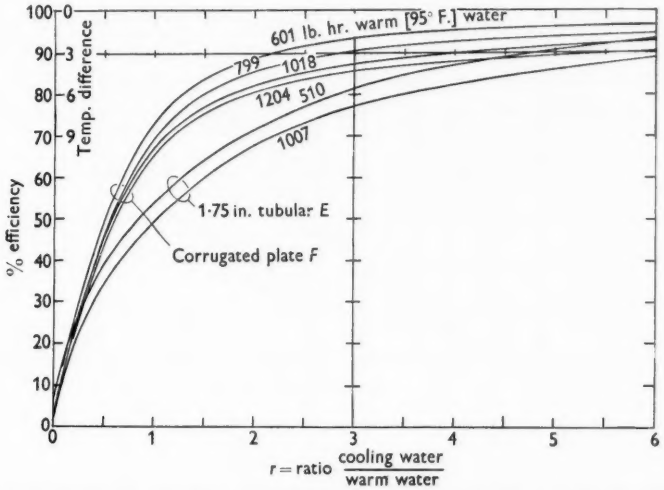

Fig. 5. Cooling efficiencies for various outside flows for a range of cooling-water ratios.

(3) The efficiencies of the corrugated plate cooler were higher than those of the tubular coolers for low rates of cooling-water flow. This characteristic is important when a cooler is installed at a farm where only a small amount of cooling water is available. The efficiency was more affected by outside flow conditions than was the case for any tubular cooler tested. This indicates that the controlling limitation on the transfer of heat was the resistance to heat flow from the warm water to the metal wall of the plate. Up to a cooling-water flow of about 3400 lb./hr. the cooling-water remained in the stream-line flow condition which was, in fact, the main cause of the high efficiencies at low cooling-water flows. It is widely claimed for these coolers that the small gap between the plates produces turbulent flow but this statement is erroneous.

(4) The efficiencies of the cooler with the spaces between the tubes filled in were intermediate between those of the tubular and corrugated plate coolers, the rate of heat transfer to the cooling-water being the controlling influence at low cooling-water flows, but at high flows the heat transfer rate of the warm water was the controlling influence.

(5) Usually a tall cooler of short width is more efficient than a cooler of equal area but having a shallow depth and large width. This effect is more pronounced in tubular coolers than for corrugated plate coolers. It is worth noting that the tall, narrow width cooler requires cooling-water with a greater pressure than the shallow depth cooler.

PART II. COOLING-WATER PRESSURE REQUIREMENTS

The pressure or head required to force cooling-water through a farm milk cooler can be of considerable importance. A milk cooler requiring a large water pressure to achieve acceptable efficiency would be of little value at a farm where the water pressure available was small.

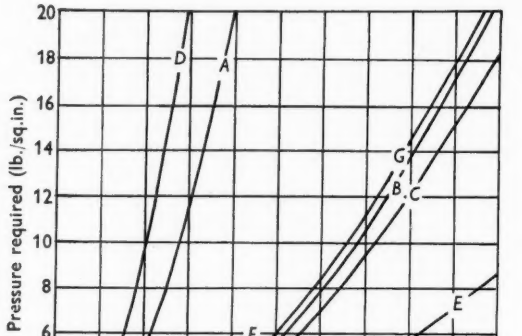

Fig. 6. Cooling-water pressure loss for flow rates up to 10,000 lb./hr.

During the present investigation the pressure difference of the cooling-water between the inlet and outlet was measured for various rates of flow. The results given in Fig. 6 are expressed in pounds per square inch. To convert pounds per square inch to feet head of water, multiply by 2·31. The figures given are for the pressure required to cause the desired flow, the total pressure being slightly in excess of these figures by the extra head caused by the elevation of the outlet above the water inlet.

By far the greater portion of the pressure loss occurs in the headers where the water reverses suddenly. The pressure loss in the headers is proportional to the square of the rate of flow and the pressure loss in the tubes is proportional to a power less than 2. The combined pressure loss is thus proportional to a power slightly less than 2, the actual figure depending on the size and length of tubes, the design of the tube headers and the smoothness of the internal surfaces. From the characteristic curves shown in Fig. 6 it can, for instance, be shown that for coolers *B*, *C*, *E* and *G* the pressure loss is proportional to rate of flow raised to the 1·86 power. For convenience, the expressions can be approximated to a square law:

$$\text{Pressure loss (lb./sq. in.) per tube} = K\ (\text{rate of flow in 1000's lb./hr.})^2,$$

where values of K are given in col. 7 of Table 5 and are valid for the coolers tested over the usual working range of flows.

Since the major part of the pressure loss is caused by water reversals in the tube headers it might appear logical to express the pressure loss as per reversal instead of

per tube, there being one less reversal than the number of tubes. However, there are three other losses associated with the tubes; at the cooler inlet, at the cooler outlet, and the friction loss in the tubes. Dividing the total pressure loss by the number of tubes rather than the number of reversals gives a more accurate figure for the pressure loss at each reversal.

It is more usual to express pressure losses in conduit systems as equivalent to a number of velocity heads—a velocity head being the head required to produce the actual velocity and given by the expression $\frac{(\text{velocity in ft./sec.})^2}{64{\cdot}4}$. The approximate number of velocity heads per tube (S) based on tube velocity is given in column 6 of Table 5.

Table 5 also shows the total and per tube pressure loss for each cooler at a cooling-water flow of 3000 pounds per hour.

Table 5. *Cooling water pressure requirements for farm milk coolers*

Code letter (1)	Type (2)	Number of tubes (3)	Pressure drop at 3000 lb./hr. (lb./sq. in.) (4)	Pressure drop per tube at 3000 lb./hr. (lb./sq. in.) (5)	S = number of velocity heads per tube (6)	K (7)
A	Tubular	9	10·2	1·13	17·50	0·125
B	Tubular	9	1·9	0·21	3·92	0·028
C	Tubular	9	2·1	0·22	3·50	0·025
D	Tubular	11	22·0	2·00	32·20	0·232
E	Tubular	5	0·9	0·18	7·85	0·022
F	Corrugated plate	—	3·0	—	—	—
G	Tubular	Top 8	1·30	0·16	Top 1·2	0·020
		Bottom 8	1·02	0·13	Bottom 0·9	0·015
		Total 16	2·32	0·145	Average 1·05	0·0175

Discussion of results

The most pertinent fact arising from the results shown in Fig. 6 or Table 5 is that both coolers *A* and *D* had an unusually high pressure loss. These abnormal conditions were due to faulty castings in which the water path was partially blocked. The trouble was traced to a faulty batch of castings supplied to the manufacturer of the coolers. As has been explained in Part I the increased turbulence caused by this partial blockage promoted a slightly improved cooling capacity.

Neglecting the above abnormal results of coolers *A* and *D*, Table 5 shows that irrespective of the size (i.e. cross-sectional area) of the tubes the pressure loss per tube did not vary considerably. This result was not expected and may fortuitously be caused by the different constructions of the tube headers. Thus the cooling water in the $1\frac{1}{4}$ and $1\frac{3}{4}$ in. pear-shaped tubing had an approximate pressure loss per tube of

$$P = 0{\cdot}023 \left(\frac{\text{flow lb./hr.}}{1000}\right)^2 \text{lb./sq. in.},$$

and in the 1 in. circular tube with a small lip, approximately,

$$P = 0{\cdot}017 \left(\frac{\text{flow lb./hr.}}{1000}\right)^2 \text{lb./sq. in.}$$

Alternatively, one can use the figures given in column 5 for a flow of 3000 lb./hr. and proportion for any other flow. Thus for a flow of 1000 lb./hr. of cooling-water the pressure loss may be expected to be about one-ninth that at 3000 lb./hr.

The number of velocity heads varied from 0·9 to 7·85. By way of comparison it may be noted that the flow resistance in velocity heads of a 180° close return bend is 1·5–2·5, and for a fully open globe valve is 5–15.

The rate of increase of pressure required for an increase of flow in the corrugated plate cooler, *F*, was not as rapid as for the tubular coolers because streamline flow in a smooth passage gave rise to a very different set of flow conditions.

SUMMARY

The main types of surface milk coolers were tested using water at 95° F. instead of milk. The coolers were tested: (*a*) for cooling efficiency at the nominal rated flow and a range of cooling-water flows; (*b*) for allowable flow at 3:1 cooling-water and cooling to within 3° F. of the cooling-water; (*c*) for the allowable flow (lb./hr. per square foot of cooling surface) for a range of cooling-water flows; (*d*) for the cooling-water pressures for various rates of flow.

The results showed that no cooler reached the required efficiency at a cooling-water ratio of 3:1 or less, the temperature difference at the bottom of the cooler varying from 3·3 to 7° F. for normal coolers.

The allowable flow per square foot of cooling surface varied from 33·8 to 114 lb./hr. when cooling to 3° F. difference with 3:1 water. The corrugated plate design had higher allowable capacity at low rates of flow of cooling-water than the tubular designs. Atmospheric cooling was small.

At 3000 lb./hr. of cooling-water the pressure loss was 3 lb./sq. in. for the corrugated plate cooler, and for the tubular coolers the pressure loss per tube was approximately 0·2 lb./sq. in. The pressure loss for the tubular coolers varied approximately proportional to the square of the rate of flow.

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520. THE EFFECT OF FEEDING IODINATED CASEIN AND L-THYROXINE UPON THE HEALTH, REPRODUCTION AND YIELD OF A DUAL-PURPOSE BREED OF CATTLE AND A PURELY DAIRY BREED

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(With 1 Figure)

The stimulating effects on milk yield of substances containing thyroxine, e.g. iodinated casein, have been extensively demonstrated in experiments carried out at various experimental stations throughout the world. These related particularly to an enhanced yield during the period of treatment, the period of treatment being timed to take place during the declining phase of lactation. The results, conclusions, and problems which these experiments yielded were very fully analysed and discussed by Blaxter *et al.* (1), who concluded that at that time 'no information was available on the effects of iodinated protein on the herd life of the cow or on general health and breeding efficiency' since almost all experiments reported had been of relatively short duration.

Van Landingham *et al.* (2), in a preliminary communication on an experiment with seven cows, suggested the possibility of impaired fertility after prolonged feeding of thyroprotein during one lactation. After several months of feeding on a maximum dose of 20 g. thyroprotein per day the cows were in thin condition and had lost body weight. Reece (3) reported no undesirable effects, except loss of body weight in three cows, when 10 g. or 15 g. thyroprotein were fed per head per day for periods of 3–19 months to 9 cows. Thomas & Moore (4) found that twelve cows fed thyroprotein for three successive lactations, from 50 days post-partum to 90 days before the next parturition, gave a smaller increase in milk production for the first 50 days of the second and third lactations than the controls. Treatment did not appear to affect parturition or the condition of the calves. Dyrendahl (5), in Sweden, found that the feeding of thyroprotein over a long period to cows and pigs caused serious damage to the liver and other organs.

The purpose of this investigation was to determine the effect of feeding iodinated casein (changed to L-thyroxine towards the end of the experiment) for 15 weeks during the declining phase of successive lactations on the health, reproduction, and yield of (*a*) a dual purpose breed of cattle (Red Poll), and (*b*) a purely dairy breed (Ayrshire). The dual purpose breed was introduced because it was thought that a fleshier animal would be able to sustain a loss of body weight with possibly less damage to its general health. The experiments started in the autumn of 1946.

EXPERIMENTAL

Animals

Twenty Red Poll heifers and twenty Ayrshire heifers were divided into two main groups as follows:

Group I	A	10 Red Polls	Controls
	B	10 Ayrshires	
Group II	A	10 Red Polls	Treated
	B	10 Ayrshires	

Because the Red Polls had to be purchased from outside sources and were difficult to obtain their calving dates were spread over a considerable period. All animals were paired according to the date of calving and allocated to the control and treated groups by the toss of a coin. At the suggestion of Dr F. Yates the groups were managed as two separate 'herds'. That is to say, when an animal became uneconomic for any reason such as injury, chronic mastitis, etc., she was culled and replaced by a heifer of similar breed.

Feeding

(*a*) The basal ration (maintenance and production) was the normal one for the main herd and consequently varied in its main constituents with the season. During the period of stall feeding it was based on the standards recommended by the Ministry of Agriculture and Fisheries (Bulletin no. 42). During the summer months grazing was *ad lib.*

(*b*) The amount of iodinated casein to be fed per animal daily was fixed at 20 g., but this amount was gradually reduced during the last 3 weeks of treatment. The 20 g. iodinated casein was incorporated in 4 lb. dairy feed cubes containing 2·6 lb. starch equivalent and 0·68 lb. digestible protein. The potency of the various batches of iodinated casein used was estimated in assays on *Xenopus* tadpoles[6,7] and it was found that 20 g. of the iodinated casein was equivalent to about 80 mg. L-thyroxine.

(*c*) The period of treatment began 16 weeks after parturition, i.e. at the beginning of the 17th week of lactation, and was continued over 15 weeks—12 weeks on 20 g. iodinated casein/day, 1 week on 15 g., 1 week on 10 g., and the final week on 5 g. During the first 12 weeks of the treatment period both the treated and control animals received 4 lb. dairy cubes daily, with or without iodinated casein, over their production requirements, and during the final 3 weeks, 3, 2 and 1 lb. respectively.

(*d*) In January 1950, L-thyroxine (80 mg.) replaced iodinated casein (20 g.) in terms of equivalent potency.

Records

(1) *General health.* Records of general health—incidence of mastitis, tuberculosis, milk fever, etc.—were made.

(2) *Reproduction.* All records of services and other relevant information were noted.

(3) *Body weights.* All animals were weighed on 3 consecutive days before treatment and at the end of 12 and 15 weeks of treatment.

(4) *Milk yield.* (*a*) Yield was weighed daily for 7 days before treatment, during treatment and for 7 days after treatment, and once per week during the remainder of the lactation. (*b*) Percentage butter-fat was estimated by the Gerber method during the periods of daily weighing.

RESULTS

Health

Table 1 shows the incidence of disease in the two breeds and in the two herds, and Table 2 the total disposals and reasons for disposal.

Table 1. *Incidence of diagnosed disease*

Group	No. of animals	No. of cases				
		Mastitis	T.B.	Milk fever	Abortion	Genital disorders*
Control Ayrshires	12	6	1	3	1	4
Treated Ayrshires	13	5	2	3	3	7
Control Red Polls	15	8	2	0	2	10
Treated Red Polls	13	5	1	2	2	8
Control herd	27	14	3	3	3	14
Treated herd	26	10	3	5	5	15

* Includes vaginitis, pyometra, cystic ovary, irregular oestrus, etc.

Table 2. *Reasons for disposal*

Group	No. of animals	Mastitis	T.B.	Sterility	Other causes*	Total disposals
Control Ayrshires	12	0	1	0	1	2
Treated Ayrshires	13	0	2	0	2	4
Control Red Polls	15	2	2	2	1	7
Treated Red Polls	13	3	1	1	0	5
Control herd	27	2	3	2	2	9
Treated herd	26	3	3	1	2	9

* Includes: one treated Ayrshire developed peritonitis after an operation to remove wire and died; one control Red Poll died after birth of 3rd calf (uterine prolapse); one treated Ayrshire sold fat (uterine prolapse); one control Ayrshire developed nephritis.

There was no significant difference between herds or breeds in the number of cases of mastitis, tuberculosis, abortion and genital disorders, or in the number of disposals due to these or other causes. Calf mortality up to 90 days of age was higher among the Red Polls than among the Ayrshires, but there was no difference between herds. The stockmen who have attended the cows since the experiment began are strongly of opinion that except during the period of iodinated casein treatment there have been no differences in general health or condition between the two herds.

Reproduction

Table 3 shows the number of services per conception. Figures for each year are given separately in order to show trends. Interpretation of the data is complicated by the high incidence of genital disorders in both herds (Table 1). There was no significant difference between herds in the number of services required per conception. Conception rates showed an improvement in the latter stages of the experiment. The low conception rate of the control Red Polls was due to one animal which required nine services before she conceived.

The number of services per conception is not an accurate measure of fertility as no account is taken of heat periods missed. A better measure is the calving index or the

Table 3. *Average number of services per conception*

Group	2nd conception		3rd conception		4th conception		5th conception		Mean all services
	Services	Cows	Services	Cows	Services	Cows	Services	Cows	
Control Ayrshires	1·20	10	1·80	10	1·00	10	1·50	8	1·36
Treated Ayrshires	1·58	12	2·45	11	1·55	9	1·60	6	1·84
Control Red Polls	2·90	14	2·63	8	2·00	4	1·00	4	2·46
Treated Red Polls	2·09	11	1·60	10	1·28	7	1·42	7	1·65
Control herd	2·20	24	2·15	18	1·28	14	1·33	12	1·85
Treated herd	1·82	23	2·04	21	1·43	16	1·53	13	1·73

interval between calvings. This is shown in Table 4. Here again there was an improvement in the later stages in all groups. The exceptionally long intervals between first and second calvings in the Red Polls can, in part, if not wholly, be accounted for by the fact that they were inoculated with strain 19 vaccine against contagious abortion after they had

Table 4. *Average interval between calvings*

Group	1st–2nd calving		2nd–3rd calving		3rd–4th calving		4th–5th calving		Mean no. of days per calf—all lactations	No. of full-time calves
	Days	Cows	Days	Cows	Days	Cows	Days	Cows		
Control Ayrshires	456	10	463	10	360	9	401	9	422	38
Treated Ayrshires	455	12	506	10	404	10	380	6	443	38
Control Red Polls	518	14	428	9	421	3	364	3	464	29
Treated Red Polls	523	11	402	10	366	7	493	6	450	34
Control herd	493	24	446	19	375	12	391	12	440	67
Treated herd	488	23	455	20	388	17	437	12	446	72

calved for the first time, and breeding was postponed for this reason for 8 weeks. The high figures in the other groups are comparable with the low breeding efficiency of the farm herd as a whole between 1946 and 1949.

Differences between the two herds in terms of the mean number of days required to produce a calf were not significant.

Body weight

The weight change between the beginning of the treatment and 12 weeks later was measured for the first lactation of each animal of the original groups when treatment occurred during the winter period and under controlled feeding. An analysis of the figures showed that the treated Ayrshires lost weight and the controls gained in weight over the period. The difference between the groups was significant at the 1% level. Both groups of Red Polls gained weight, the control animals more than the others ($P < 0{\cdot}02$). These results are illustrated in Table 5. In subsequent lactations the body-weight figures are not so comparable or reliable, because the treatment occurred sometimes during stall feeding when food intake was controlled and sometimes during the grazing season.

Table 5. *Average gain in weight (lb.) during treatment period for all animals in their first lactation*

	Control	Treated	S.E. of difference
Ayrshires	46·2	−18·6	±19·8
Red Polls	68·2	13·2	±22·0

In Tables 5–11, a difference must be approximately 2·15 times its S.E. for significance at the 5% level, and 3·00 times its S.E. for significance at the 1%. The 5% level is indicated by one asterisk, the 1% by two.

Milk yield

It was found after the first year that the pairing adopted did not lead to increased accuracy. Consequently, the yield was analysed thereafter as if the animals had been completely randomized. The analysis was greatly simplified by this, since, for various reasons, animals had to be discarded and the identity of the pairings could not be maintained. All replacements used were heifers, since it was not possible for administrative reasons to carry spares from the beginning of the experiment.

The features investigated for each of the four lactations were the milk yield, the butter fat yield and the length of lactation.

The measures of milk yield analysed were: (1) the milk yield during the period of treatment; (2) the total milk yield from the commencement of treatment until the end of lactation; (3) the average daily milk yield from the beginning of treatment until the end of lactation. For the purpose of analysing these yields, all data from animals whose daily yield did not reach 20 lb. at some period during the pre-treatment period (i.e. in the first 16 weeks of lactation) were discarded, as it was considered that such animals would not normally be retained in a dairy herd.

Table 6. *Milk yield (lb.) for pre-treatment period*

	Control (C)	Treated (T)	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	3538	3035	503**	±169
Lact. 2	4598	4038	560	±288
Lact. 3	4776	4171	605*	±216
Lact. 4	4901	4093	808*	±283
Red Polls				
Lact. 1	3111	2989	122	±199
Lact. 2	3454	3346	108	±364
Lact. 3	4126	3831	295	±392
Lact. 4	3838	4047	−209	±577

In Table 6 is shown the milk yield for the pre-treatment period. It is unfortunate that, although the allocation to groups was at random, the control Ayrshires turned out on average to be better milkers than those due to receive iodinated casein. All subsequent comparisons were made in the light of this difference, and where a relationship was found to exist with the pre-treatment yield, it was taken into account.

In Table 7 is shown the milk yield for the duration of treatment. For both breeds, there was a significant regression of yield during treatment upon pre-treatment yield

Table 7. *Milk yield (lb.) for period of treatment*

	Control	Treated	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	2304	2387	−83	±136
Lact. 2	2600	2594	6	±159
Lact. 3	2421	2687	−266	±208
Lact. 4	2539	2533	6	±204
Red Polls				
Lact. 1	1959	2285	−326*	±151
Lact. 2	1919	2046	−127	±168
Lact. 3	2208	2330	−122	±396
Lact. 4	2447	2342	105	±422

($P < 0{\cdot}01$). For every pound that the yield of an animal was above the average of its group in the pre-treatment period, its yield would be expected to be 0·53 lb. above its group average for the duration of treatment if it were an Ayrshire, and 0·84 lb. above the average if a Red Poll.

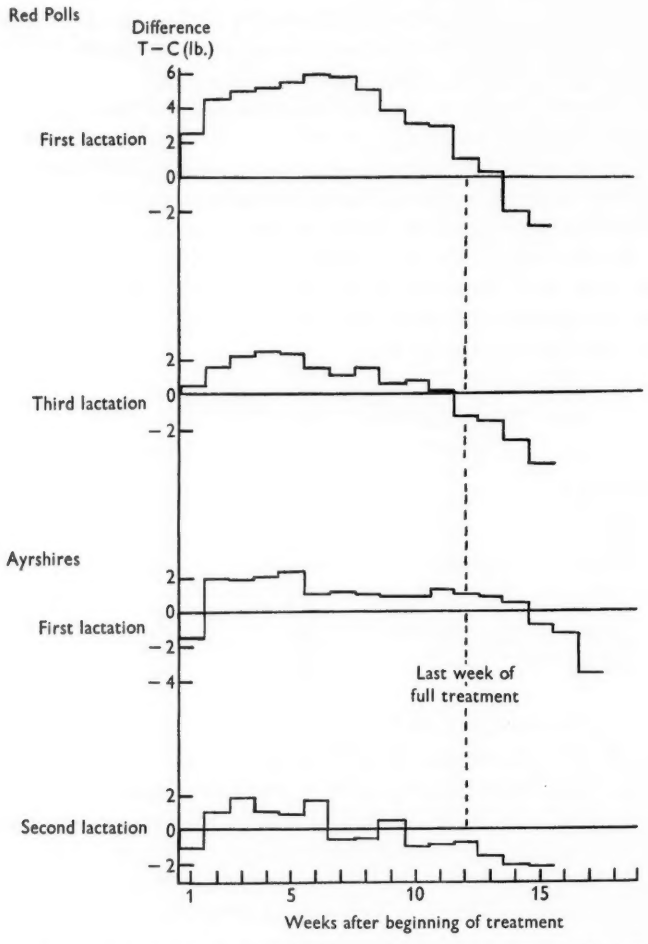

Fig. 1. Difference between daily milk yield of treated and control groups, in lb. (adjusted for difference in pre-treatment yield).

There was, however, a temporary response to treatment, and this is illustrated in Fig. 1, where the daily difference in milk yield between treated and control animals is shown for two lactations of the Red Polls and two of the Ayrshires. These figures were averaged for each week from the beginning of treatment after adjusting for differences in pre-treatment yield. There was always an initial benefit from the use of iodinated casein, which reached its maximum 4–6 weeks after the beginning of treatment, and gradually became less until the control animals were yielding more than the treated ones. This was in agreement

with the results of Table 9 which shows that the control animals had a longer lactation in the instances illustrated in Fig. 1.

Table 8 shows the total yield from the commencement of treatment until the end of lactation after adjustments were made for differences in pre-treatment yield. There were no significant differences between treated and control animals, either for Ayrshires or Red Polls, but it is interesting to note that the control Ayrshires had a slightly greater

Table 8. *Milk yield (lb.) from beginning of treatment until end of lactation*

(Adjusted for differences in pre-treatment yield.)

	Control	Treated	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	4834	4121	713	±580
Lact. 2	4316	3825	491	±612
Lact. 3	3641	4374	−733	±878
Lact. 4	3244	3685	−441	±624
Red Polls				
Lact. 1	4253	3336	917	±660
Lact. 2	3560	2609	951	±575
Lact. 3	3820	3022	798	±728
Lact. 4	3592	3400	192	±768

Table 9. *Length of lactation in weeks*

	Control	Treated	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	48·9	41·2	7·7*	±3·5
Lact. 2	44·2	37·6	6·6	±3·2
Lact. 3	38·6	39·5	−0·9	±3·3
Lact. 4	36·6	35·0	1·6	±2·6
Red Polls				
Lact. 1	48·3	37·4	10·9*	±4·6
Lact. 2	42·3	33·2	9·1*	±4·0
Lact. 3	40·6	32·7	7·9*	±3·5
Lact. 4	37·0	37·0	0·0	±4·2

yield than the treated animals for the first two lactations and the control Red Polls for the first three. This showed agreement with the length of lactation recorded in Table 9. For the first two lactations of the Ayrshires and all but the last lactation of the Red Polls, the treatment appeared to shorten the lactation ($P < 0{\cdot}05$ in each case). The final measure of milk yield which was analysed was the average yield from the commencement of treatment until the end of lactation. This was found to be positively correlated with the pre-treatment yield. Table 10 shows this average yield, adjusted for differences in pre-treatment yield. It was only in the first lactation of the Red Polls that the treated animals had a higher average yield ($P < 0{\cdot}01$), and it may be possible to account for this by the fact that before their first lactation the Red Polls carried more flesh than the Ayrshires and this extra reserve was available for conversion to milk. After this there was the constant strain of successive lactations on the animal preventing it from again building up a comparable reserve.

It was found all through the experiment that butter-fat yield reflected milk yield and to illustrate this the percentage of fat in the milk for the duration of treatment is given in Table 11. There were no significant differences in this variable, and in fact tables showing the butter-fat yield would be virtually a repetition of the milk-yield data.

Table 10. *Average daily milk yield (lb.) from the beginning of treatment until the end of lactation*

(Adjusted for differences in pre-treatment yield.)

	Control	Treated	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	21·7	21·9	−0·2	±1·3
Lact. 2	22·9	23·6	−0·7	±1·3
Lact. 3	24·5	24·3	0·2	±1·9
Lact. 4	24·7	24·6	0·1	±2·4
Red Polls				
Lact. 1	18·4	22·4	−4·0**	±1·2
Lact. 2	18·6	21·1	−2·5	+1·4
Lact. 3	21·5	22·9	−1·4	±4·3
Lact. 4	22·7	23·8	−1·1	±4·1

Table 11. *The percentage of butter-fat in the milk during the treatment period*

	Control	Treated	Difference (C – T)	S.E. of difference
Ayrshires				
Lact. 1	3·76	3·90	−0·14	±0·19
Lact. 2	3·74	4·03	−0·29	±0·17
Lact. 3	3·84	3·93	−0·09	±0·14
Lact. 4	3·79	3·57	0·22	±0·20
Red Polls				
Lact. 1	3·27	3·47	−0·20	±0·20
Lact. 2	3·43	3·51	−0·08	±0·20
Lact. 3	3·63	3·44	0·19	±0·20
Lact. 4	3·54	3·52	0·02	±0·11

DISCUSSION

Health

The primary purpose of this experiment was to determine the effects of feeding iodinated casein or L-thyroxine on the health of a dual purpose breed and a dairy breed, when given over successive lactations. In order to ensure that the test was thorough, treatment was given for 15 weeks in each lactation—12 weeks at the maximum dose of 20 g./head/day decreasing to 15, 10 and 5 g. in the ensuing 3 weeks. That the material was potent is shown by the characteristic rise in milk yield during the earlier weeks of treatment. That the period of treatment selected was adequate to admit the maximum effects of the dosage was demonstrated by the decline in yield before the end of treatment. Despite the above steps to ensure adequate treatment there were no adverse effects on the health of animals receiving iodinated casein apart from a temporary reduction in the rate of body-weight gain, which in some cases resulted in a loss. This loss of body weight may have been an early sign of damage but the long intervening periods between treatments (over 300 days) were sufficient to allow complete recovery. The fact that the hormonal effect had diminished about half way through the treatment indicated a natural reaction of the organism to offset the excessive drain caused by the enhanced yield. Such a natural compensating effect might operate at all except excessive levels of administration. It should be noted that due to poor fertility in both the treated and control groups the interval of recovery was longer than might often be encountered. Nevertheless, several of the treated cows had comparatively short intervals between calvings and showed no undue signs of strain or ill-health.

Lactation performance

As a consequence of the essential plan of this experiment, to test the effect of iodinated casein on health, the treatment was carried beyond the point of the most efficient use of iodinated casein at the level selected. As was earlier indicated, this level was based on the maximum safe dosage for use over a prolonged period as judged by previous work, because there might in practice be a tendency to push and prolong treatment.

At the end of each lactation the treated and control groups had performed equally well. There was, due to iodinated casein, an enhanced yield which rose to a maximum between 4 and 5 weeks from the commencement of treatment. This was followed by an accelerated reduction in yield of those animals receiving iodinated casein, so that by the end of the treatment period the animals receiving iodinated casein had not produced more milk than the controls. The one exception to this was the effect of the treatment upon Red Polls in their first lactation, where there was a net balance at the end of treatment in favour of the treated animals. It was considered possible that this exception was due to the Red Polls being fleshier before their first lactation, so that there was an extra reserve available for conversion to milk. After this, the constant strain of successive lactations may have prevented the build-up of a comparable reserve.

Fig. 1 shows three typical examples and the one exception. This last was the effect of treatment on the milk yield of the Red Polls for their first lactation, where there was a greater elevation of yield than in any other lactation. This effect reached its peak at the sixth week of treatment and fell away, initially, more slowly than in the other lactations. Thus in the first lactation of the Red Polls the effect of treatment was to enhance the milk yield for the duration of treatment, and also the average daily milk yield from the beginning of treatment until the end of lactation (Tables 7 and 10). This effect was offset, however, by the other effect which iodinated casein appeared to have, namely a tendency to reduce the persistency of lactation (Table 9), so that there was no difference between the total yields of the two subgroups, treated and control Red Polls, in their first lactation.

As illustrated in Table 11, the percentage of butter-fat in the milk did not change with the addition of iodinated casein to the diet, so that where milk yield was elevated, there was a corresponding improvement in butter-fat yield.

It may be concluded that if iodinated casein is fed at a level which is not higher than the level used in this experiment any adverse effect upon the health and reproduction of cows is unlikely to result. On the other hand, the level of treatment used did not lead to any overall increase in milk yield, although a temporary stimulation was observed. It is possible that some other system of feeding iodinated casein might have led to an overall benefit, but it was not the purpose of this experiment to determine whether this was so. Whether the farmer should feed iodinated casein at the level tested above would depend partly upon whether he wanted to obtain an enhanced yield in mid-lactation, or wished the yield to be spread over the longer period as happened in both control subgroups described above.

SUMMARY

1. Iodinated casein or L-thyroxine, under the conditions of this experiment, had no adverse effect on the general health of a dual purpose (Red Poll) or a dairy breed (Ayrshire) of cattle.

2. The treatment did not increase the total milk or butter-fat production.

3. The treated animals did significantly better than the controls in one lactation only and in the following variables only: (*a*) daily milk yield adjusted for pre-treatment differences—Red Polls, lact. 1; (*b*) milk yield for duration of treatment adjusted for pre-treatment differences—Red Polls, lact. 1.

4. The control animals had in four lactations a significantly longer lactation than the treated animals.

5. There was a temporary stimulus due to the treatment, but the resulting increase in yield was not maintained over the whole lactation and had indeed disappeared before the end of the treatment period.

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521. NORMAL VARIATIONS IN THE RATE OF MACHINE MILKING

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(With 5 Figures)

INTRODUCTION

The variations in machine milking rate have been described in outline in a number of papers. Several workers have shown that each cow has an individual pattern of milking which varies little during lactation and even over much longer periods (1,2,3,4,5). Many of these studies give results from selected groups of cows, and no figures are available on the range and distribution of the variations which are likely to occur in a herd of cows. Although Whittleston did not find a lactation trend in milking rate (4), other workers agree that rate declines with advance in lactation (3,6,7). Up to the present, no one has investigated the reasons for this lactation trend nor attempted to measure the effect of increase in lactation age (i.e. age in lactations) on milking rate.

The object of this paper is to fill some of these gaps in our knowledge of machine milking rate. As far as possible the results have been analysed statistically in order to give definite measures of the effects discussed, and it is hoped that they will be amplified at a later date when more records are available.

Source of data

Apart from the records concerned with lactation trends which were obtained in the Ayrshire herd of a neighbouring farmer, the data used for the analyses presented in this paper were collected between 1946 and 1952 in the herd of the National Institute for Research in Dairying. The N.I.R.D. herd consists of Shorthorn, Guernsey and Friesian cows, the former two breeds relying on home-bred stock for replacements. Except for those cows on experiment the herd is managed in a similar way to a typical dairy herd in the area, and for details of general feeding and management reference should be made to another paper where Bailey (8) has described this in some detail.

In the research herd milking-rate records were not obtained from cows that were on any experiment likely to affect rate of milking. The measurements of milking rate were made at three consecutive morning milkings for each cow in each lactation, and in order to eliminate stage of lactation effect these records were made in the fifth and sixth weeks after calving. This period was chosen since it is usually the time of highest daily milk yields. If, for an obvious reason, at one morning milking an abnormal milking-rate record was obtained, a further reading was taken on the following morning.

Method of recording

There are several ways of measuring the machine milking rate of dairy cattle, varying in complexity from a simple measurement of the duration of milking to the record made by the intricate mechanism designed and constructed by Whittleston (9). For any detailed

study, it is necessary to record milking rate throughout milking and for the purposes of this study the simple yet efficient method described by Foot(2) has been used. This technique of suspending the milking machine bucket on a spring balance, and recording the balance reading at half-minute intervals is a simple method, little elaborate equipment is needed and with an accurate spring balance the errors are small. Nevertheless, it has the disadvantage that the records are not continuous, the rate being recorded at half-minute intervals. It is also necessary to accustom the cows to this method of recording before representative readings can be obtained and care is needed because movements of the cow tend to alter the balance readings.

In detail, the method used was as follows. All cows were accustomed to the routine of preparation for milking by washing with warm water within 2 min. of the start of milking. Feeding was completed some time before milking commenced and, as a result, the cows were not conditioned to accept feeding as a stimulus to milk ejection. The zero reading was taken when two teat cups were placed on the teats, and from that time the spring balance records were noted at half-minute intervals until the weight of milk entering the pail in one half-minute period fell to 0·3 lb. or less. At this point the regular cowshed operator began to machine strip and continued to do so until he considered that all the strippings had been removed. Half-minute readings were not obtained during the period of machine stripping because the normal manipulation of the teat cup assembly caused abnormal vibration of the balance finger.

A Gascoigne bucket-type milking machine fitted with moulded rubber liners of a standard pattern (Gascoigne standard roundtop) was used throughout the study. The pulsator which gave a pulsation ratio of 1:1 was adjusted to operate at forty-eight pulsations per minute. The vacuum in the main pipe-line was maintained at 15·0 in. Hg by a weight-loaded regulator.

RESULTS

Methods of simplification of records and the definitions used

From 1946 to 1952 records were obtained from 141 cows. Since most of these cows were recorded in more than one lactation a total of 326 records was collected. Except where otherwise stated, the whole of these data has been used in the analysis presented below.

There are two simple graphical methods of illustrating the milking-rate records collected; one is by plotting the quantity of milk obtained against the time taken and the other by plotting rate of milking against time. These two methods for identical records are given in Fig. 1 for a fast milker and a slower milker. The quantity/time graphs illustrate the pattern of milking rate which all cows follow. There is an initial period of accelerating milking rate, a period when milking rate reaches its peak and finally there is a period of decline in rate of milk flow. Although all cows follow this general pattern the duration of these three periods and the rate of milking during the periods differ greatly from cow to cow. Since it has not been found practicable to devise a single measure of the milking rate of each cow the method adopted has been to extract figures which measure milking rate in the various phases. The extracted results for individual cows can be used in comparisons and analyses. The following are the definitions of terms used in the analyses:

Rate in first minute (lb.)—amount of milk removed from the udder during the first minute of milking.

Mid-milking rate (lb./min.)—rate of milking in the second and third minutes of milking.

Peak flow (lb./min.)—maximum milking rate attained during milking.

Machine rate (lb./min.)—mean rate of milking up to the time when machine stripping commenced.

Overall rate (lb./min.)—mean rate of milking over the whole milking period.

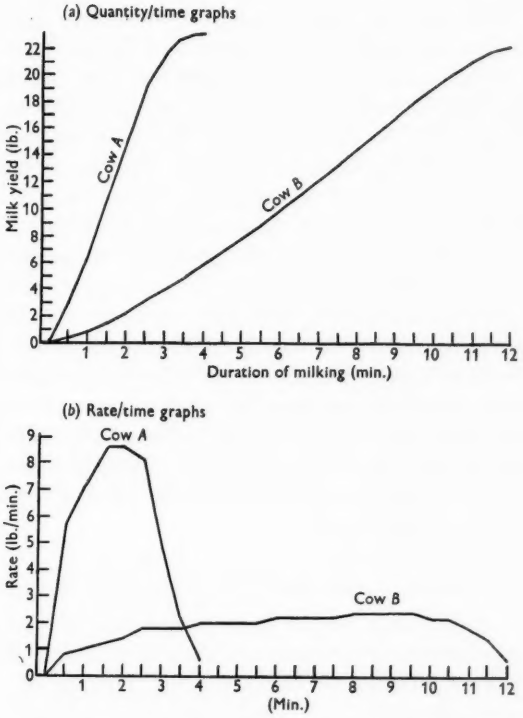

Fig. 1. Rate/time and quantity/time graphs; two ways of illustrating milking rate.

Stripping yield (lb.)—yield of machine stripping.

Stripping time (min.)—duration of machine stripping.

Machine time (min.)—duration of machine milking up to the start of machine stripping.

Total time (min.)—complete duration of the milking and stripping.

Morning milk yield (lb.)—total milk yield at the milking when recordings were made.

While yield and duration of stripping are not strictly measures of milking rate, they have been calculated because they have an important bearing on the total duration of milking.

Records of four breeds of cows were collected, but because of the relatively small numbers of cows in each breed, a detailed comparison of the milking rates of the four breeds has not been made. Examination of the data, however, shows that the same range of values was found in each of the four breeds.

Day-to-day variations in machine milking rate

Although it has been shown that the main factor determining milking rate is the effective size of the teat orifice during milking (10), there are day-to-day variations even under the best management conditions. In the following analysis two methods of measuring these variations have been used. The coefficients of variation of the different measures of milking rate have been calculated from records collected from ten cows at fourteen consecutive morning milkings. Since the mean drop in morning milk yield over this period was only 0·05 lb./cow, no correction for lactation trend was made. The second method was to calculate the coefficients of variation of the same factors for each of ten cows recorded at weekly intervals over a complete lactation. Because of the obvious lactation trends the coefficients of variation of each of the factors are those from linear regression on morning milk yield and not between the individual readings.

The coefficients calculated by these two methods are very similar and are given in Tables 1 and 2. In each case the milking-rate determination with the smallest coefficient of variation is that for peak flow, where values of 8·05 and 7·48% have been obtained. These values are only a little higher than the coefficient of variation of morning milk yield for the ten cows over the 14-day period. The machine rate and mid-milking rate showed slightly greater variations than peak flow. Since the rate in the first minute is affected by the regularity of the milk ejection mechanism it is not surprising that the variation of this factor is higher than any of the other true measures of milking rate. The relatively marked variability of stripping yield and duration is evident from the fact that their coefficients of variation ranged from 36 to 53% (Table 1).

These results indicate that day-to-day variations in the true measures of milking rate for a particular cow are relatively small as long as the milking routine is constant and correction is made for lactation trends.

Variations between morning and evening milkings

Matthews *et al.* (6) and Dodd & Foot (3) have given details of differences between the milking rates of cows recorded at morning and evening milkings. Both reported faster milking after the longer interval, although the total duration of milking was higher because of the greater milk yield.

Table 3 and Fig. 2 show the various measures of milking rate of six cows recorded at morning and evening milking on six consecutive days (i.e. $a.m._1$ and $p.m._1$) and also at separate morning milkings later in the same lactation ($a.m._2$). The recordings in later lactation were made when the morning yield was approximately the same as afternoon milk yield ($p.m._1$) in early lactation. Thus, the mean milk yields of the six cows at the milkings were 25·1, 17·1 and 17·5 lb. respectively for the $a.m._1$, $p.m._1$ and $a.m._2$ milkings. The quantity/time graph in Fig. 2 illustrates milking rate for only 3 min. because some cows had finished milking in that time.

The $a.m._1$ and $p.m._1$ records confirm earlier findings. While the rate in first minute, mid-milking rate, peak flow and machine rate are greater at the morning milking, the total time is longer, because of the much higher milk yield.

Although $p.m._1$ and $a.m._2$ records were obtained when yields were virtually the same, the rate of milking was greater in early lactation afternoon milkings ($p.m._1$), and consequently total time was less.

Table 1. *Coefficients of variation of a number of measures of machine milking rate calculated from records collected at fourteen consecutive morning milkings*

Cow ...	Bridget	Campion 20	Charis	Dido	Dorrit 14	Honor	Ivy	Rosalie 30	Sarah 2	Winsome 24	Mean
Rate in first min.	4·42	18·01	8·56	16·33	8·23	10·81	12·03	10·96	14·06	10·06	11·35
Mid-milking rate	4·91	7·10	7·21	12·32	4·48	5·74	12·40	9·08	12·06	6·53	8·18
Peak flow	4·15	10·77	9·31	10·14	7·30	6·03	6·23	7·01	13·18	6·42	8·05
Machine rate	6·20	10·97	7·91	13·72	9·31	11·60	5·53	7·06	10·59	8·59	9·15
Machine time	5·95	13·96	6·06	11·86	10·28	14·08	10·67	7·49	9·63	11·00	10·10
Stripping yield	40·64	66·03	96·00	32·80	28·96	78·52	76·60	29·34	49·60	31·00	52·95
Morning milk yield	3·13	4·85	2·92	6·48	4·23	6·26	10·11	5·35	5·42	6·76	5·55
Total time	6·49	10·31	9·27	9·26	12·78	9·99	9·02	12·50	6·81	13·74	10·02
Stripping time	25·71	41·74	61·88	27·49	38·04	50·06	29·92	19·63	42·80	28·11	36·54
Overall rate	7·27	8·84	9·26	14·76	10·27	10·08	8·68	7·65	8·96	10·77	9·65

Table 2. *Coefficients of variation of measurements of machine milking rate calculated from the linear regressions on morning milk yield throughout a complete lactation*

Cow ...	Brownie	Buttercup	Etonia	Frantic	Isobel	Kate	Nuisance	Pearl	Primula	Rose	Mean
Rate in first min.	15·62	8·94	26·39	15·74	12·91	6·21	12·23	5·99	11·49	7·94	12·35
Mid-milking rate	8·93	8·17	13·59	10·95	8·54	5·55	9·28	6·53	12·24	6·82	9·06
Peak flow	9·25	7·90	10·44	8·77	6·10	3·95	8·60	3·33	9·50	6·92	7·48
Machine rate	7·01	9·99	16·29	9·63	5·68	5·18	8·72	3·80	12·24	7·15	8·57
Machine time	11·92	11·17	9·53	11·27	5·03	6·40	8·46	5·04	12·05	10·38	9·12
Stripping yield	22·54	20·73	47·11	53·90	66·85	33·60	19·82	29·04	56·65	33·02	38·33

Table 3. *The difference between milking rate at morning and evening milkings*

	Rate in first min. (lb./min.)			Mid-milking rate (lb./min.)			Peak flow (lb./min.)			Machine rate (lb./min.)			Total time (min.)		
Cow	a.m.$_1$*	p.m.$_1$*	a.m.$_2$*	a.m.$_1$	p.m.$_1$	a.m.$_2$	a.m.$_1$	p.m.$_1$	a.m.$_2$	a.m.$_1$	p.m.$_1$	a.m.$_2$	a.m.$_1$	p.m.$_1$	a.m.$_2$
A	6·63	6·23	6·40	6·94	5·89	5·55	7·38	7·07	6·90	5·89	5·60	4·89	5·25	4·88	4·75
B	2·43	2·50	2·43	3·28	3·22	3·00	3·70	3·48	3·37	3·57	2·81	2·42	9·25	6·17	6·72
C	3·33	3·28	2·70	3·93	3·79	2·94	4·33	3·98	3·38	3·65	3·23	2·62	8·83	7·46	8·96
D	7·67	7·45	5·36	8·42	4·23	4·67	8·88	7·69	5·50	6·78	5·08	4·18	5·42	4·71	5·08
E	7·00	5·53	4·23	7·04	5·94	4·99	7·85	7·38	7·18	5·53	5·47	4·61	5·33	4·54	4·94
F	3·70	3·57	2·77	4·54	3·66	3·18	4·77	4·33	4·57	3·70	3·28	2·86	6·63	4·79	4·88
Mean	5·13	4·76	3·98	5·69	4·46	4·06	6·15	5·66	5·15	4·85	4·25	3·60	6·79	5·43	5·89

* a.m.$_1$, the mean of six records obtained at six consecutive morning milkings in early lactation; p.m.$_1$, the mean of six records taken at the afternoon milkings following the mornings recorded for a.m.$_1$; a.m.$_2$; the mean of records taken at morning milkings in later lactation when morning milk yield was the same as at the evening milkings used for p.m.$_1$.

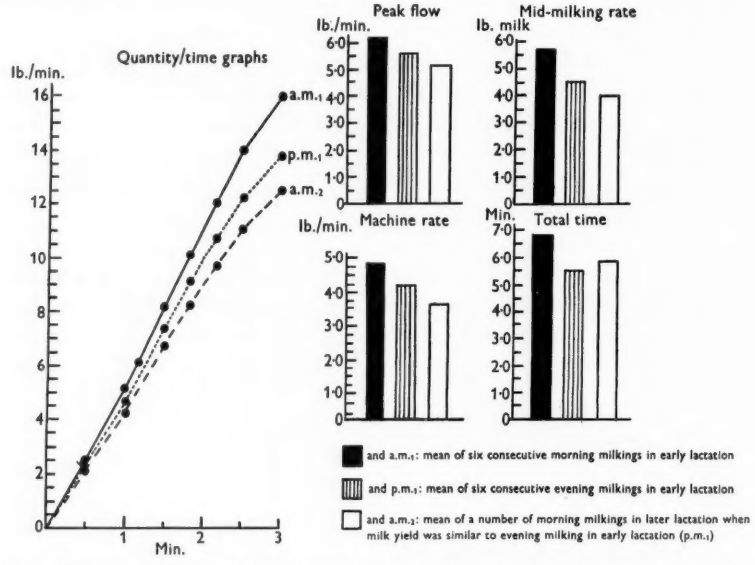

Fig. 2. The differences between machine milking rate at morning and evening milkings (mean of six cows).

In a later section it is shown that the lactation trend in milking rate can be divided into two components, one of which is related to yield and the other to the interval since calving. The details above give a good demonstration of these two components. The difference in milking rate between a.m.$_1$ and p.m.$_1$ is related to the differences in yield and between p.m.$_1$ and a.m.$_2$ to the different intervals since calving.

The differences between the milking rates of cows within a herd

Table 4 shows the range and distribution of the various measures of milking rate classified for each age group and for the total of 326 lactation records. The histograms in Fig. 3 illustrate the same distributions for all cows.

The total time varied from 4·42 to 21·50 min.; only 2·8% of the records were under 5 min. and 8·3% over 14 min. About 20% of all records were between 6·0 and 6·9 min.

Table 4. *The distribution of measures of machine milking rate*

(1) Total time (min.). Range 4·42–21·50 min.

Lact. No.	Less than 5	5·0–5·9	6·0–6·9	7·0–7·9	8·0–8·9	9·0–9·9	10·0–10·9	11·0–11·9	12·0–12·9	13·0–13·9	Over 14·0
1	3	14	18	9	14	8	7	1	5	2	5
2	2	13	25	15	8	9	7	4	4	1	8
3	1	6	13	9	8	10	5	5	2	0	5
Over 4	3	7	9	11	10	6	12	6	7	0	9
Total	9	40	65	44	40	33	31	16	18	3	27
%	2·76	12·27	19·94	13·49	12·27	10·12	9·51	4·91	5·52	0·92	8·28

(2) Rate in first minute (lb./min.). Range 0·27–9·90 lb./min.

Lact. no.	Less than 0·9	1·0–1·9	2·0–2·9	3·0–3·9	4·0–4·9	5·0–5·9	6·0–6·9	7·0–7·9	8·0–8·9	Over 9·0
1	7	19	14	23	16	4	3	0	0	0
2	1	11	18	18	19	16	9	2	2	0
3	1	5	15	13	11	9	7	1	2	0
Over 4	0	8	16	13	11	10	12	3	4	3
Total	9	43	63	67	57	39	31	6	8	3
%	2·76	13·19	19·32	20·55	17·48	11·96	9·51	1·84	2·45	0·92

(3) Mid-milking rate (lb./min.). Range 0·48–8·05 lb/min.

Lact. no.	Less than 0·9	1·0–1·9	2·0–2·9	3·0–3·9	4·0–4·9	5·0–5·9	6·0–6·9	7·0–7·9	Over 8·0
1	3	7	23	10	28	11	4	0	0
2	1	6	14	14	28	24	7	2	0
3	0	2	12	11	14	16	7	2	0
Over 4	0	2	11	19	22	10	12	3	1
Total	4	17	60	54	92	61	30	7	1
%	1·23	5·21	18·40	16·56	28·22	18·71	9·20	2·15	0·31

(4) Peak flow (lb./min.). Range 1·00–10·40 lb./min.

Lact. no.	Less than 2·0	2·0–2·9	3·0–3·9	4·0–4·9	5·0–5·9	6·0–6·9	7·0–7·9	8·0–8·9	Over 9·0
1	6	18	15	22	18	6	1	0	0
2	4	9	17	19	25	11	8	2	1
3	1	5	14	14	12	8	7	3	0
Over 4	0	8	19	13	13	10	9	4	4
Total	11	40	65	68	68	35	25	9	5
%	3·37	12·27	19·94	20·86	20·86	10·73	7·67	2·76	1·53

(5) Machine rate (lb./min.). Range 0·71–6·38 lb./min.

Lact. no.	0·0–0·99	1·00–1·49	1·50–1·99	2·00–2·49	2·50–2·99	3·00–3·49	3·50–3·99	4·00–4·49	4·50–4·99	Over 5·0
1	2	5	10	16	9	23	14	4	2	1
2	0	5	2	16	14	20	16	9	12	2
3	1	0	6	7	14	6	13	8	6	3
Over 4	0	1	5	15	14	12	8	9	7	9
Total	3	11	23	54	51	61	51	30	27	15
%	0·92	3·37	7·06	16·56	15·64	18·71	15·64	9·20	8·28	4·60

(6) Overall rate (lb./min.). Range 0·70–5·44 lb./min.

Lact. no.	0·50–0·99	1·00–1·49	1·50–1·99	2·0–2·49	2·50–2·99	3·00–3·49	3·50–3·99	4·00–4·49	Over 4·50
1	2	6	13	19	25	12	9	1	0
2	1	4	10	16	24	17	14	7	2
3	1	3	6	11	15	15	6	4	3
Over 4	0	3	13	15	14	12	11	9	3
Total	4	16	42	61	78	56	40	21	8
%	1·23	4·91	12·88	18·71	23·92	17·18	12·27	6·44	2·45

Table 4 (*cont.*)

(7) Yield of stripping (lb.). Range 0·10–6·40 lb.

Lact. no.	Less than 0·30	0·30–0·59	0·60–0·89	0·90–1·19	1·20–1·49	1·50–1·79	1·80–2·09	2·10–2·39	2·40–2·69	Over 2·70
1	15	33	15	13	6	0	3	0	0	1
2	14	15	16	20	15	2	4	3	2	5
3	3	7	12	13	9	5	1	6	2	6
4 and over	1	6	12	13	11	6	4	6	4	17
Total	33	61	55	59	41	13	12	15	8	29
%	10·12	18·71	16·87	18·10	12·57	3·99	3·68	4·60	2·45	8·89

(8) Stripping time (min.). Range 0·22–7·23 min.

Lact. no.	Less than 0·49	0·50–0·99	1·00–1·49	1·50–1·99	2·00–2·49	2·50–2·99	3·00–3·49	3·50–3·99	4·00–4·49	Over 4·50
1	9	42	21	10	4	0	0	0	0	0
2	2	28	35	19	7	3	1	0	1	0
3	0	13	25	13	10	0	0	0	1	2
4 and over	0	14	21	21	15	3	2	1	2	1
Total	11	97	102	63	36	6	3	1	4	3
%	3·37	29·75	31·28	19·32	11·04	1·84	0·92	0·31	1·24	0·92

and 80% were less than 10·9 min. From the records it appears that these cows have much slower milking rates than those recorded by other workers (5, 11). However, it should be borne in mind that the data used for this analysis are not selected, and that the records were obtained at morning milkings when the cows were giving their highest milk yield, which is the time when the duration of milking is at its maximum.

The rate in the first minute of milking varies from 0·27 to 9·90 lb./min. with approximately 20% of the records between 3·0 and 3·9 lb./min. and nearly 60% of all values within the limits 2·0 and 4·9 lb./min.

The 326 peak flow records varied from 1·00 to 10·40 lb./min. Only 3·4% of the values were below 2 lb./min. and 1·5% above 9·0 lb./min. with nearly 60% of the records within the limits 3·0 and 5·9 lb./min.

The mid-milking rate showed a similar distribution to peak flow with 80% of the values falling between 2·0 and 5·9 lb./min.

Machine rate and overall rate had a similar range of values; the former was 0·71–6·38 lb./min. and the latter 0·70–5·44 lb./min. The class intervals with the greatest number of records were 3·00–3·49 lb./min. for machine rate and 2·50–2·59 for overall rate; 18·7 and 23·9% respectively of the values were in these two class intervals.

Yield of machine stripping showed a wide range from 0·10 to 6·40 lb.; over half of the values fell between 0·30 and 1·19 lb. per milking. This was reflected in the range for the duration of stripping which was from 0·22 to 7·23 min. with 60% falling between 0·50 and 1·49 min.

The coefficients of variation of each of these separate determinations of machine milking rate were calculated for all the records collected in each age group, and these are given in Table 5. In order to compare the variations between cows with the day-to-day variations of individual animals, the coefficients of variation in Table 2 can be compared with those in Table 5. Whereas the *within-cow* coefficients calculated over the whole lactation for total time, rate in the first minute, mid-milking rate, peak flow, machine rate, overall rate and stripping yield are 10·02, 11·35, 8·18, 8·05, 9·15, 9·65 and 52·95%

respectively, the coefficients of variation of these same measures *between eighty-six first-lactation animals* are 36·52, 47·81, 38·74, 35·07, 32·29, 30·49 and 74·59% respectively.

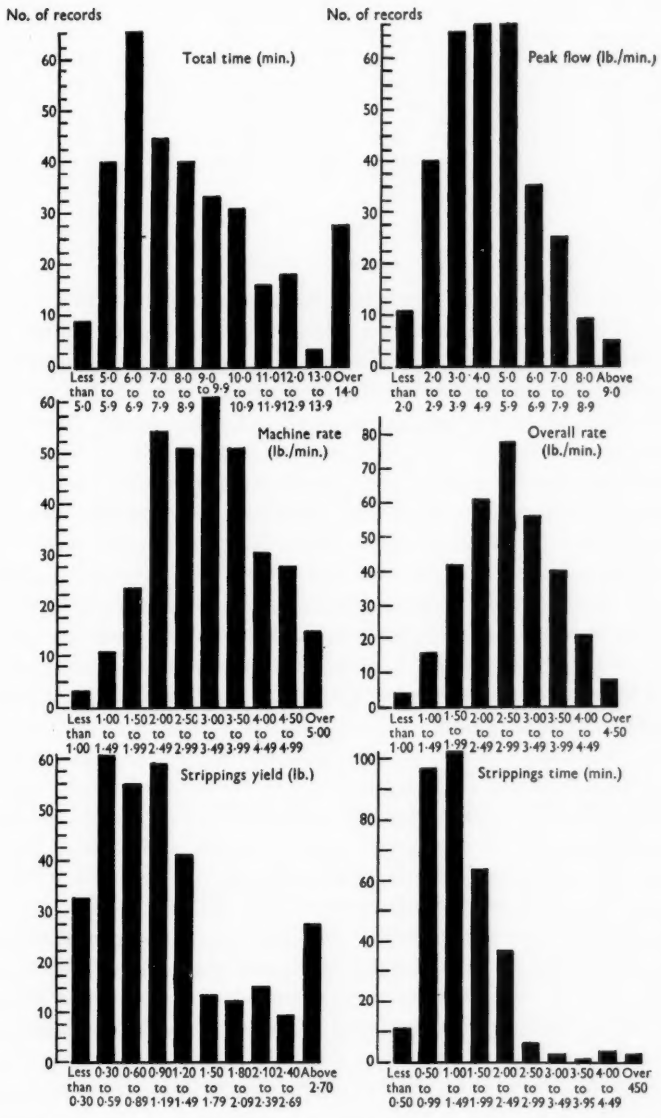

Fig. 3. Histograms illustrating the distribution of 326 records of various measures of machine milking rate.

This comparison illustrates very clearly that between-cow differences are very much greater than within-cow variations. This is apparent not only for the true measures of milking rate but also for stripping yield, although here the effect is not so marked.

Table 5. *The coefficients of variation of measures of milking rate, calculated for all cows in each lactation age group*

	No. of records	Morning milk yield (%)	Total time (%)	Rate in first min. (%)	Mid-milking rate (%)	Peak flow (%)	Machine rate (%)	Overall rate (%)	Stripping yield (%)	Stripping time (%)
1st lactation	86	19·25	36·52	47·81	38·74	35·07	32·29	30·49	74·59	59·82
2nd lactation	96	20·59	39·53	42·65	34·07	33·79	30·10	28·99	73·44	48·15
3rd lactation	64	18·97	31·31	44·18	33·49	35·45	32·01	31·12	74·49	59·51
4th lactation	29	19·25	35·92	50·91	35·15	39·29	36·72	33·46	85·84	49·65
5th lactation	26	17·15	32·21	42·86	27·43	34·48	31·18	30·00	57·54	40·85
6th, 7th, 8th, 9th and 10th lactations	25	17·53	34·74	51·77	35·60	41·65	36·01	35·40	49·96	53·67

Lactation trends

In order to measure lactation trends in milking rate a separate series of measurements was made with ten cows in the herd of a neighbouring farmer. The milking rates of these cows were determined at weekly intervals for a complete lactation. The particular milking machine and level of vacuum used in this herd was the same as that in the herd of the main study, while the cows in this case were of the Ayrshire breed. In order to condense the considerable amount of data collected the lactations were divided into 21-day periods, and the three readings obtained in each period averaged to give a single value. For the nine cows with lactations of over 36 weeks the mean lactation changes over the twelve 21-day periods were calculated for each of the milking-rate measurements. These means, presented graphically in Fig. 4, indicate that there were lactation changes in milking rate. As milk yield increased in early lactation and then slowly decreased, so the yield in first minute, mid-milking rate, peak flow, machine rate and overall rate showed similar trends. In spite of the downward trend in milking rate with advance in lactation, the duration of milking decreased because the decline in yield was proportionately greater than the decline in milking rate.

The linear regression coefficients of milking rate on morning milk yield for the ten cows are given in Table 6. These regressions for rate in first minute, mid-milking rate, peak flow, machine rate and machine time are all positive, and of the fifty regression coefficients thirty-eight are significant at the 1% level and three at the 5% level of significance. The regression coefficients of stripping yield on milk yield indicate that in this group there is no relationship between these values.

Further investigations revealed that the lactation trends in milking rate were not solely due to the changes in milk yield which took place. From Fig. 4 it will be seen that during the time of spring grazing (i.e. periods 7–9) milk yield was constant yet peak flow and mid-milking rate continued to decline. In order to obtain more information on this 'stage of lactation' effect, partial regression coefficients were calculated. These coefficients measure the relative effects during lactation of morning milk yield (x_1) and lactation stage (x_2) on peak flow (y_1), machine rate (y_2) and machine time (y_3) (the lactation stage being the number of 21-day periods since calving). These coefficients are given in Table 7.

It will be seen that the partial regression coefficients of lactation stage and morning milk yield on peak flow, machine time and machine rate, calculated on the mean values of the nine cows, are significant, as are most of the coefficients for the individual cows. These

results indicate that as morning milk yield declines, the peak flow, machine rate and machine time decline even when allowance is made for a stage of lactation effect. Also, that as the interval since calving increases, the peak flow and machine rate decreases and the machine time increases independently of yield changes.

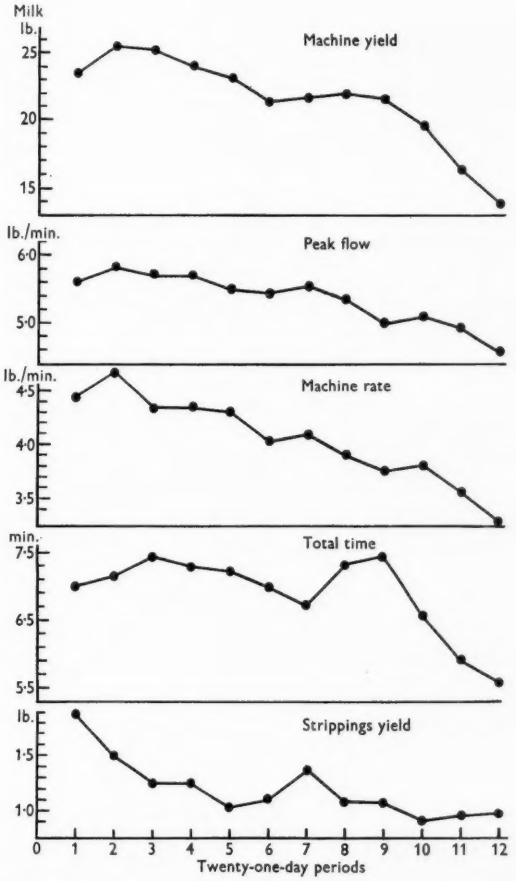

Fig. 4. Within-lactation variations in machine milking rate (mean of nine cows).

In order to find out whether the rate of decline in milking rate which occurs with advance in lactation is related to the initial milking rate of the animal, further calculations were carried out. A comparison has been made between the mid-milking rates of the nine cows in early lactation (during the week when milk production was at its greatest) with the mid-milking rate in later lactation when milk production had fallen to 60% of the initial value. These results are illustrated in Fig. 5*a* where the fall in mid-milking rate of the nine cows is plotted against the initial rate and in Fig. 5*b* where the percentage fall is plotted against initial rate. Clearly the absolute and relative decline in milking rate with fall in yield is greater in the case of the fast milking cows.

Table 6. *The linear regression coefficients of milking rate on morning milk yield, together with their standard errors calculated for ten cows over complete lactations*

	Nos. of 21-day periods	Peak flow	Mid-milking rate	Machine rate	Machine time	Rate in first min.	Stripping yield
Brownie	15	+0·029±0·046	+0·186±0·039**	+0·137±0·024**	+0·056±0·049	+0·145±0·055*	−0·098±0·040*
Buttercup	13	+0·302±0·040**	+0·365±0·037**	+0·210±0·036**	+0·088±0·024**	+0·274±0·042**	−0·002±0·011
Etonia	13	+0·034±0·021	+0·030±0·023	+0·028±0·017	+0·304±0·040**	+0·029±0·019	+0·122±0·039**
Frantic	12	+0·061±0·017**	+0·033±0·018	+0·055±0·015**	+0·174±0·055**	+0·011±0·015	+0·064±0·048
Isobel	13	+0·055±0·011**	+0·066±0·014**	+0·057+0·012**	+0·225±0·018**	+0·047±0·019*	+0·060±0·038
Kate	12	+0·225±0·019**	+0·265±0·025**	+0·186±0·028**	+0·051±0·016**	+0·168±0·026**	+0·029±0·015
Nuisance	15	+0·209±0·031**	+0·280±0·030**	+0·200±0·024**	+0·043±0·018*	+0·118±0·037**	−0·017±0·015
Pearl	12	+0·090±0·023**	+0·252±0·029**	+0·139±0·019**	+0·082±0·019**	+0·045±0·035	+0·024±0·024
Primula	12	+0·095±0·019**	+0·086±0·023**	+0·081±0·017**	+0·177±0·051**	+0·071±0·017**	+0·025±0·043
Rose	9	+0·099±0·015**	+0·117±0·013**	+0·082±0·015**	+0·205±0·040**	+0·076±0·012**	+0·032±0·040

* Significant at the 5% level. ** Significant at the 1% level.

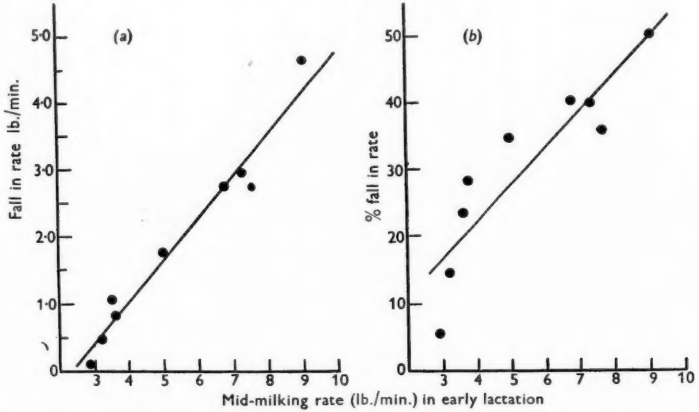

Fig. 5. The relationship between the lactation decline in milking rate and initial milking rate.

Table 7. *Lactation changes in milking rate*

x_1 = morning milk yield (mean milk yield (lb.) at the times of recording). x_2 = stage of lactation (the number of the three-weekly periods after calving).

Cow	$byx_{1\cdot2}$	$byx_{2\cdot1}$
(a) Partial regression coefficients of morning milk yield and stage of lactation on peak flow		
(1) Brownie	+0·1136±0·062	+0·0611±0·034
(2) Buttercup	+0·2607±0·058**	−0·0548±0·053
(3) Etonia	+0·0333±0·034	−0·0014±0·036
(4) Frantic	−0·0085±0·026	−0·1180±0·038**
(5) Isobel	+0·0589±0·017**	+0·0057±0·019
(6) Kate	+0·3068±0·033**	+0·1120±0·048*
(7) Nuisance	+0·1477±0·035**	−0·0890±0·035*
(8) Pearl	+0·0987±0·034*	+0·0103±0·030
(9) Primula	+0·0176±0·025	−0·1316±0·036**
(10) Rose	+0·0172±0·026	−0·1682±0·049**
Mean†	+0·0537±0·021*	−0·0629±0·021**
(b) Partial regression coefficients of morning milk yield and stage of lactation on machine rate		
(1) Brownie	+0·1711±0·036**	+0·0243±0·016
(2) Buttercup	+0·1662±0·053**	−0·0576±0·048
(3) Etonia	+0·0197±0·028	−0·0123±0·030
(4) Frantic	−0·0083±0·022	−0·1072±0·033**
(5) Isobel	+0·0606±0·013**	+0·0050±0·014
(6) Kate	+0·2269±0·053**	+0·0549±0·066
(7) Nuisance	+0·1559±0·028**	−0·0649±0·028*
(8) Pearl	+0·1050±0·025**	−0·0414±0·022
(9) Primula	+0·0057±0·018	−0·1268±0·026**
(10) Rose	+0·0209±0·024	−0·1263±0·045*
Mean†	+0·0412±0·019*	−0·0739±0·018**
(c) Partial regression coefficients of morning milk yield and stage of lactation on machine time		
(1) Brownie	−0·0907±0·050	−0·1067±0·027**
(2) Buttercup	+0·1239±0·034**	+0·0464±0·031
(3) Etonia	+0·3798±0·057**	+0·1047±0·060
(4) Frantic	+0·4155±0·067**	+0·4069±0·099**
(5) Isobel	+0·2380±0·028**	+0·0187±0·031
(6) Kate	+0·0408±0·042	−0·0150±0·058
(7) Nuisance	+0·0814±0·022**	+0·0546±0·022*
(8) Pearl	+0·1136±0·027**	+0·0366±0·024
(9) Primula	+0·4020±0·059**	+0·3770±0·089**
(10) Rose	−0·0062±0·072	−0·4355±0·137*
Mean†	+0·2867±0·030**	+0·1582±0·030**

† Calculated from mean values of cows (1) to (9).

Lactation age

In Table 8 the 326 lactation records of milking rate are classified according to lactation age. Since all the data were used in the preparation of this table the records of a particular cow may have been used in the calculation of only one or of more than one of the lactation means.

It appears from this table that with increase in age there is a gradual increase both in yield and milking rate. Nevertheless, the total duration of milking increases because the increase in yield is progressively greater than the increase in rate. These trends are also shown in the table of milking rate distributions (Table 4). The second part of Table 8 gives the individual increases and decreases between lactations, together with their significance. The trends are gradual and, apart from the changes between the first and second lactations, are rarely significant at the 5% level. However, for all the determinations measured the overall changes between the first lactation group and the oldest lactation group are significant at either the 5 or 1% level.

Table 8

Lactation	No. of cows	Morning milk yield (lb.)	Total time (min.)	Rate in 1st min. (lb./min.)	Mid-milking rate (lb./min.)	Peak flow (lb./min.)	Machine rate (lb./min.)	Overall rate (lb./min.)	Stripping yield (lb.)	Stripping time (min.)	Daily yield (lb.)	Max. daily yield (lb.)	Days† in milk
(*a*) Mean values of various measures of milking rate classified according to lactation age													
1st lactation	86	19·47	8·50	3·03	3·53	4·04	2·85	2·52	0·67	1·08	32·13	34·16	37·7
2nd lactation	96	22·79	8·73	3·99	4·25	4·89	3·29	2·87	1·05	1·33	38·29	39·42	40·1
3rd lactation	64	23·61	8·82	4·04	4·45	4·97	3·37	2·90	1·48	1·59	39·42	43·67	48·5
4th lactation	29	25·21	9·44	4·41	4·40	5·18	3·40	2·94	1·46	1·57	41·52	45·66	43·6
5th lactation	26	24·44	8·99	4·60	4·48	5·29	3·43	2·93	1·62	1·65	40·35	44·21	42·2
6th lactation and above	25	25·88	9·88	4·72	4·55	5·38	3·41	2·89	2·13	2·13	44·42	46·06	37·3
Mean		23·57	9·06	4·13	4·28	4·96	3·29	2·84	1·40	1·56	—	—	—
(*b*) The differences between the lactation mean values given in (*a*)													
Between 1st and 2nd lactations		+3·32**	+0·23	+0·96**	+0·72**	+0·85**	+0·44**	+0·35**	+0·38**	+0·25**	—	—	—
Between 2nd and 3rd lactations		+0·82	+0·09	+0·05	+0·20	+0·08	+0·08	+0·03	+0·43**	+0·26**	—	—	—
Between 3rd and 4th lactations		+1·60	+0·62	+0·37	−0·05	+0·21	+0·03	+0·04	−0·02	−0·02	—	—	—
Between 4th and 5th lactations		−0·77	−0·45	+0·19	+0·08	+0·11	+0·03	−0·01	+0·16	+0·08	—	—	—
Between 5th and 6th lactations		+1·44	+0·89	+0·12	+0·07	+0·09	−0·02	−0·04	+0·51	+0·48	—	—	—
Between 1st and 6th lactations		+6·41**	+1·38*	+1·69**	+1·02**	+1·34**	+0·56**	+0·37*	+1·46**	+1·05**	—	—	—

† Interval between calving and the start of milking rate recording.

Elsewhere (12) we have shown that in this herd the lactation age at culling is related to milking rate in such a way that slow milkers are culled at an earlier age than fast milkers. Thus the figures in Table 8 do not give a true measure of the effect of age on milking rate. In order to obtain a less biased estimate of the effect of age a separate analysis was carried out with the milking rate data from thirty-four cows recorded in their first, second and third lactations. The partial regression coefficients of morning milk yield (x_1) and lactation age (x_2) on peak flow (y_1), stripping yield (y_2) and machine rate (y_3) were calculated and are as follows:

(*a*) peak flow
$$by_1x_1.x_2 = +0{\cdot}0725 \pm 0{\cdot}0282^{**},$$
$$by_1x_2.x_1 = +0{\cdot}0069 \pm 0{\cdot}1725;$$

(*b*) strippings yield
$$by_2x_1.x_2 = +0{\cdot}0113 \pm 0{\cdot}0207,$$
$$by_2x_2.x_1 = +0{\cdot}1979 \pm 0{\cdot}1265 \ (t = 1{\cdot}56);$$

(*c*) machine rate
$$by_3x_1.x_2 = +0{\cdot}0744 \pm 0{\cdot}0172^{**},$$
$$by_3x_2.x_1 = -0{\cdot}1550 \pm 0{\cdot}1050 \ (t = 1{\cdot}48).$$

From this series of partial regression coefficients it will be seen that the effect of morning milk yield on peak flow and machine rate, independent of change in lactation age, is significant at the 1 % level of significance, whereas the effect of age independent of change in yield is not significant. This means that with these cows the changes in milking rate that take place with advance in age are a reflexion of the changes in yield that occur. Neither of the partial regression coefficients of yield and age on stripping yield are significant at the 5 % level.

Since it has been shown (see below) that the total duration of milking is related to the milk yield, peak flow and stripping yield, a further more complex set of partial regression coefficients had to be calculated to find the true effect of age on total duration of milking. Thus the partial regression coefficients of morning milk yield (x_1), lactation age (x_2), peak flow (x_3) and stripping yield (x_4) on total time (y) are as follows:

(1) $byx_{1 \cdot 234} = +0{\cdot}2978 \pm 0{\cdot}0234^{**},$

(2) $byx_{2 \cdot 134} = +0{\cdot}3916 \pm 0{\cdot}1404^{*},$

(3) $byx_{3 \cdot 124} = -1{\cdot}4109 \pm 0{\cdot}0803^{**},$

(4) $byx_{4 \cdot 123} = +0{\cdot}3302 \pm 0{\cdot}1105^{**}.$

Coefficient (2) indicates that with each increase in lactation age of one lactation, independent of the effect of change in morning milk yield, peak flow and stripping yield, the total duration of milking increases by 0·4 min. Thus, in spite of the fact that peak flow is not affected by age, as a cow becomes older the duration of milking increases, presumably because of the effect of age on yield of stripping and machine rate.

Factors affecting the duration of milking

To the farmer the most important measure of the milking rate of a cow is the total duration of milking, for slow-milking cows tend to upset the general routine of milking and increase labour costs. Logically we would expect the total duration of milking to be dependent on the amount of milk given at a milking, the rate of milking and the yield of strippings which have to be removed by manipulation of the milking machine. In order to measure the relative importance of these factors the partial regression

coefficients of morning milk yield (x_1) peak flow (x_2) and stripping yield (x_3) on total time (y) have been determined using the data from eighty-six first-lactation heifers in order to eliminate the effect of age. These partial regression coefficients:

$$byx_{1\cdot23} = +0\cdot3394 \pm 0\cdot0502^{**},$$
$$byx_{2\cdot13} = -1\cdot8844 \pm 0\cdot1341^{**},$$
$$byx_{3\cdot12} = +0\cdot8907 \pm 0\cdot3646^{*},$$

show that all three factors independently influence total milking time. In order to measure the relative importance of these three factors the b-primes or standard partial regression coefficients were calculated:

$$b'yx_{1\cdot23} = 0\cdot4103,$$
$$b'yx_{2\cdot13} = 0\cdot8593$$
$$b'yx_{3\cdot12} = 0\cdot1434.$$

These b-prime values indicate that if we give the influence of stripping yield on a total time a value of unity, then morning milk yield is three times and peak flow six times more important.

The correlation between machine time and various other determinations of milking rate

Beck *et al.* (5) correlated a number of the determinations of milking rate with machine time. They found that the percentage yield obtained in the first 2 min. had a correlation coefficient with machine time of 0·92, whereas none of the other coefficients was greater than 0·6. In order to find out whether similar results would be obtained in the Reading herd a series of correlation coefficients was calculated from the records of the eighty-six first-lactation animals. These results, together with those of Beck *et al.*, are given in Table 9. The correlation coefficients of percentage yield in 2 min. with machine time are nearly identical for both series of records. However, the coefficients for rate in first minute, peak flow and machine rate, calculated from the Reading data, are much higher than those of Beck *et al.*, and in fact are very similar to the coefficient obtained for percentage yield in 2 min.

Table 9. *Correlation coefficients calculated between machine time and other measures of milking rate*

	No. of animals	Rate in first min.	Percentage yield in 2 min.	Peak flow	Machine rate	Morning milk yield	Stripping yield
A. Calculated from records of 86 heifers	86	0·827**	0·869**	0·889**	0·850**	0·113	0·064
			Starting time				
B. Records from Beck *et al.* (5)	61	0·438**	0·922**	0·566**	0·598**	0·132	0·240

No reason can be given for this difference, except that the cows used by Beck *et al.* were faster milking and lower yielding and hence would give a greater proportion of their milk in 2 min. In the case of the eighty-six heifers only 35·9% was obtained in this time. In spite of the differences between the two sets of results percentage yield in 2 min. remains a useful indirect measure of milking rate. It is closely correlated with machine time and can be obtained simply without upsetting the cow and without elaborate equipment.

DISCUSSION

Baxter *et al.* (10) demonstrated that the major factor controlling milking rate is the anatomy of the teat orifice, so it is not surprising that research work on milk rate invariably indicates that each cow has an individual pattern of milk which shows only relatively minor changes from day to day, over the lactation period and even with advance in lactation age. The results presented above amply confirm these earlier findings and, in addition, give a measure of the extent of the variations in milking rate which are to be expected within a herd.

In spite of the fact that the major variations in milking rate are between cows, there are quite definite changes and trends in the milking rates of individual cows, and the extent of these variations has been measured in the results presented above. By calculating partial regression coefficients it has been possible to throw new light on the relationship of these variations with other factors. The variations in milking rate which occur with advance in lactation age are related solely to the changes in yield which occur over this period, since age itself has no influence on milking rate. On the other hand, the variations within a lactation are related to both the changes in yield and to the increase in the interval since calving. These results, though interesting, do not explain the mechanism of the variations, although it is possible to speculate that they are due, in some way, to the changes in internal udder pressure which occur with changes in yield and stage of lactation.

With machine milking the actual force expelling the milk from the udder is the pressure difference between the milk in the udder and the vacuum of the machine. Since internal milk pressure may be equivalent to 2–3 in. Hg, variations in this factor cannot be ignored in studies of milking rate.

The day-to-day variations are more difficult to explain. They may be a reflexion of changes in the efficiency of the milk-ejection mechanism which would affect internal udder pressure, or alternatively they may be due to changes in the tonus of the sphincter muscle controlling the effective size of the teat orifice during milking.

Further work is needed on the exact way in which changes in milk yield and internal udder pressure affect milking rate, and it seems probable that the results of such work would help in designing new and improved milking machines.

SUMMARY

1. A study has been presented of the machine milking rate of 141 cows recorded in 326 lactations.

2. The coefficients of variation between cows of various measures of machine milking rate are usually in the range 30–40%, whilst the within-cow variations are of the order of 8–11%.

3. The differences between the milking rates of cows in a single herd are given, together with the distribution of these differences.

4. Milking rate declines as lactation advances, the decline being greater for fast milking cows.

5. Lactation decline in milking rate is related independently to change in milk yield and to interval since calving.

6. With thirty-four cows recorded in their first, second and third lactations the effect

of age on milking rate has been measured. Whilst increase in age independent of change in yield has no effect on peak flow, total duration of milking increases.

7. Total duration of milking is influenced more by change in peak flow than by changes in milk yield or yield of strippings.

8. A series of correlation coefficients is presented which show the relationship between machine time (i.e. duration of milking up to the start of machine stripping) and various other measures of milking rate.

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522. AUTOMATIC APPARATUS FOR DRAWING THE MILK-EJECTION CURVES OF DAIRY COWS UNDER CONTROLLED MILKING CONDITIONS

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(With 8 Figures)

An earlier article(1) described an apparatus for the plotting of milk-ejection curves of cows milked by machine. This apparatus met the requirements of (*a*) not causing any interference with normal shed procedure, (*b*) being remote controlled so that the operator could observe the response of the cow under test without being present in the bail, (*c*) being semi-automatic. Its chief deficiencies were the need for an operator and the fact that the end-point of the milking curve required the observation of a flow indicator by the milker. The apparatus to be described meets these additional requirements. The completely objective determination of the end-point of the milking process made possible by the apparatus eliminates the most important 'human error' in the accurate recording of the milk-ejection response of the cow.

It is convenient to divide the description of the apparatus into the following sections: (*a*) the transmitting weighing machine; (*b*) the recording unit; (*c*) the automatic timing and end-point determining apparatus; (*d*) The basic mechanical milker.

(*a*) *The transmitting weighing machine*

This consists of a cylinder suspended from three springs and connected to the vacuum and milk lines by means of a valve similar to that used in our milk-measuring units(2). Fig. 1 shows the general construction of the unit. The frame (*a*) made of duralumin holds the cylinder (*b*) in such a way as to be easily removable. The springs (*c*) support the frame, which is guided in its movements by the sets of three ball-bearing rollers (*d*, *e*), and the guides (*f*, *g*). Guide (*f*) terminates in a composite plate (*h*) consisting of two pairs of quadrants which may be rotated with respect to one another so that the gap between the quadrants may be varied. The plate moves in the cylinder of oil (*i*) so providing critical damping of the system. The guide (*g*) has a rack (*j*) fixed to it. This engages pinion (*k*) whose shaft carries the indicating pointer (*l*), the pulse disk (*m*) and the magnet of the 'Magnesyn' transmitter (*n*).

The valve (*o*) is similar to that described earlier(2), and connects the vacuum lead (*p*) to the top of the can via tube (*q*) during milking, admits air for mixing via tube (*r*) and finally withdraws the milk via (*r*) and takes a sample into burette (*s*). The milking machine teat cups are connected to tube (*t*).

The pulse disk (*m*) is a silver-plated brass disk round the periphery of which are 100 bakelite inserts so arranged that there are 100 equal metal and bakelite surfaces presented to the brush (*u*). The action is simply the rotation of the wheel with increasing weight causing a series of pulses to be sent to the recording unit. When needed the Magnesyn transmitter consisting of magnet (*n*) and coil (*v*) may be fitted.

(*b*) *The recording unit*

This unit consists essentially of a lever mechanism similar to that used in Trub Tauber electrical recording instruments (3), driven by a toothed quadrant actuated by a magnet and pawl. Fig. 2 gives a general picture of the unit. The driving pulses operate the electromagnet (*a*) and pawl (*b*), so pulling quadrant (*c*) round against the tension of spring (*d*). The ratchet (*e*) prevents the quadrant from returning, but is lifted from the teeth

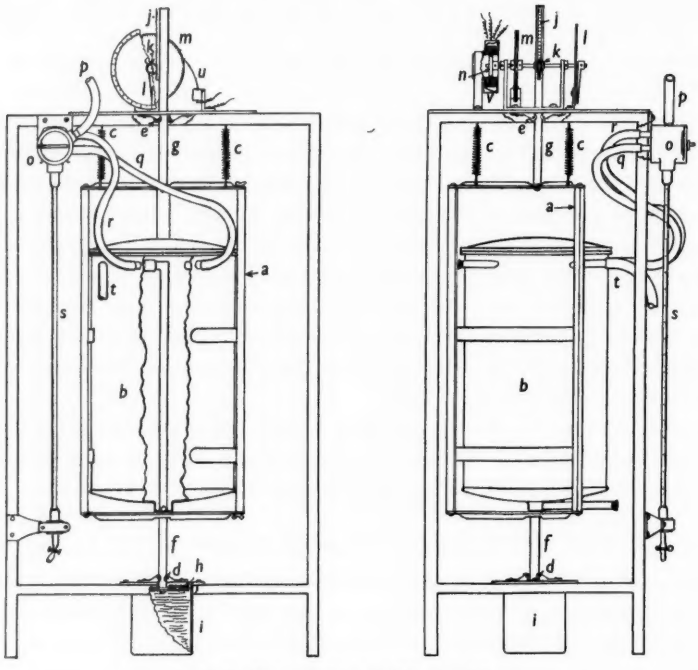

Fig. 1. Transmitting weighing machine.

during the zeroing operation by the electromagnet (*f*). At (*g*) is a micro switch which actuates the zeroing relays in the event of a curve reaching the top of the paper. The two levers (*h*) and (*i*) give a straight line over the width of the paper at the writing end. This end is fitted with the essentials of 'Biro' pen on the end of a springy portion. When the zeroing relay operates bar (*j*) lifts so raising the pen from the paper.

The graphs are drawn on $5\frac{1}{2}$ in. calculating machine paper fed from drum (*k*) between guides (*l*, *l*) and under the rubber roller (*m*) which is held by spring tension against a toothed wheel. This driving wheel is actuated by a Venner synchronous electric clock motor which is controlled by the timing relays so that the paper only moves when a curve is being drawn.

The pulses from the weighing unit are amplified by the vacuum tube relay (Fig. 3) so as to avoid any sparking on the brush at the pulse disk. This relay has a time constant of the order of $\frac{1}{50}$ sec. in order to eliminate any chatter which might be caused by mechanical vibration at the brush. This arrangement has given a reliable performance, free from spurious pulses. The circuit of the recorder driving and zeroing mechanisms is set out in

Fig. 3. Zeroing is controlled by a contact on the drain and milk control valve (*o*) (Fig. 1). When this valve is returned to the milking position it closes the 24 V. circuit of the valve position-indicating lamp and in so doing supplies a pulse of current through the condenser *C* to the relay R_1. The contacts (*a*) close for a moment so energizing the coil of relay R_2

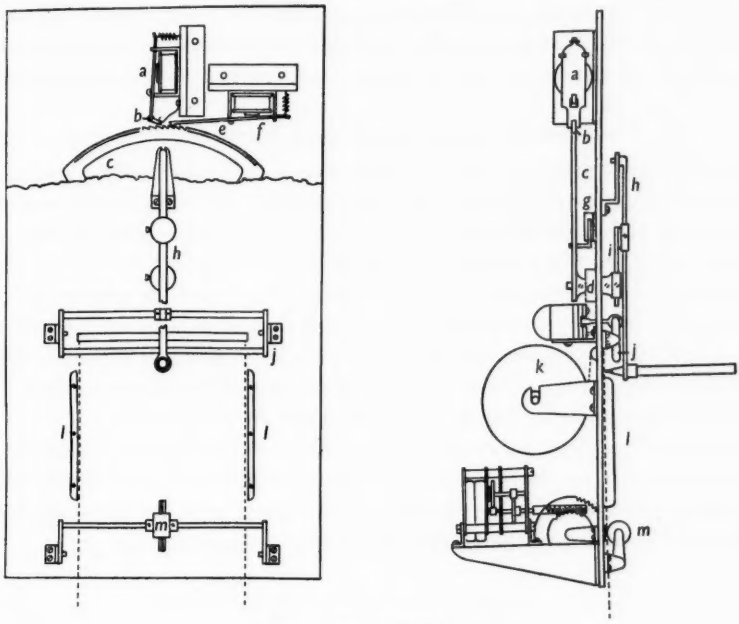

Fig. 2. Recording unit.

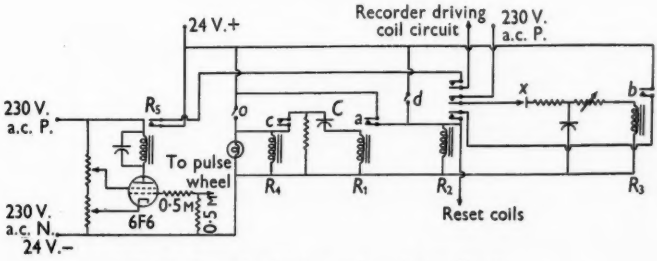

Fig. 3. Recorder driving and zeroing mechanism.

which latches itself in electrically, and in so doing opens the 230 V. a.c. circuit to the rectifier (*x*). This causes the relay system R_3 to open the contacts (*b*) (after a delay of some 2 sec.), so unlatching relay R_2. While R_2 is energized the resetting coils of the timer and recording unit are energized so that the pen is lifted from the paper and returned to zero. The timer is also reset to zero.

Relay R_4 has a larger time constant than R_1, and so opens contacts (*c*) a moment after the pulse has worked R_1. This ensures that R_1 will not operate due to the pulse caused by the opening of the indicator lamp circuit.

The pulsing circuit is disconnected by a set of contacts on R_2. This ensures that any pulses coming into the system cannot operate the recorder drive electromagnet during the zeroing operation. Such pulses will cause the driving pawl to catch the quadrant and prevent its returning to zero.

When the quadrant reaches the full-scale position a micro-switch (*d*) is closed. This energizes R_2, and causes the complete zeroing sequence to be set in train. By this means full curves are plotted for cases in which the volume of milk delivered is above the full-scale reading of the instrument and the pulsing disk has started a second revolution.

(*c*) *The automatic timing and end-point determining apparatus*

One of the greatest difficulties in work on animals is the precise definition of the conditions under which an experiment is performed so that strict comparisons can be made. The completely automatic recording of milk-ejection curves and milking times requires a definition of the point at which the milking process is complete. If this is not done the cows are subjected to variations in the use of the milking machine which could produce such effects as a progressively lengthening milking time. The point on the milk-ejection curve at which the flow rate reaches 0·5 lb./min. has been chosen as the end-point so that milking time is defined as the interval between putting on the teat cups until the point at which the flow rate has fallen to half a pound of milk per minute.

The new timer is entirely electrical and does not depend on the use of a flow-indicating sight glass as used in our earlier apparatus. The chief reason for abandoning the earlier system was the observation that with some slow milking cows the degree of frothing of the milk tends to alter the clearing time of the flow indicator.

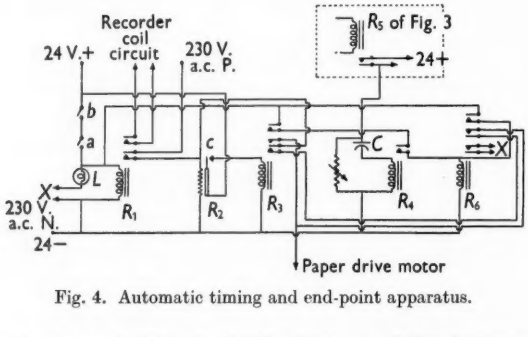

Fig. 4. Automatic timing and end-point apparatus.

The timer circuit is shown in Fig. 4. At the bottom of the dropper connecting to the teat cups of the milking machine is a micro-switch operated by a rubber diaphragm pulled in by the application of vacuum to the teat cups. This switch is shown at (*a*). The closing of the 'vacuum switch' energizes R_1. This closes a 24 V. circuit to the milking indicator lamps, completes the pulse relay circuit and closes a 230 V. a.c. circuit to the synchronous motor of the paper drive in the recorder and to the operating heater of a thermal delay relay R_2. After a delay of 1–2 min. (depending on adjustment) relay R_2 closes contacts (*c*). This energizes relay R_3, so causing the 230 V. a.c. supply to the paper drive motor to be diverted from a direct connexion to one through a pair of 'break' contacts on relay R_6. The small relay R_4 is fed by a set of contacts on the pulse relay R_5 through the condenser C. Provided that the pulses are supplied at a rate greater than

the equivalent to a milk flow of 0·5 lb./min. R_4 remains open because the initial charge on the condenser has not had time to leak to a level at which the current pulse can operate the relay. When the contacts on relay R_4 momentarily close at the end-point, R_6 is energized, thus opening the synchronous motor circuit, turning off the milking indicator lamp and closing a pair of contacts which cause the relay to latch in electrically. If a cow is to be 'machine stripped' push button (*b*) within easy reach of the milker is pressed so de-energizing R_1 for a moment and thus resetting R_6 which is again energized

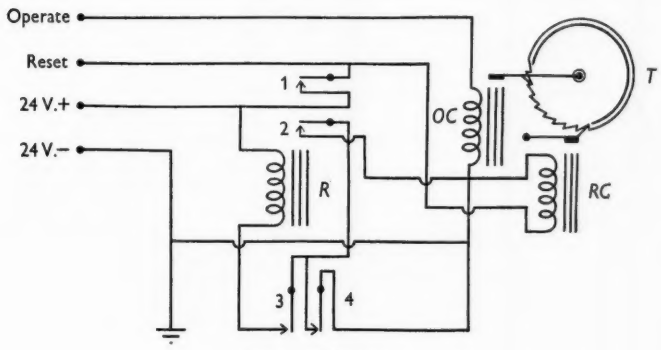

Fig. 5. Milking timer.

when the milk flow rate falls to 0·5 lb./min. Should the teat cups fall off the vacuum switch (*a*) opens and stops the time-base, etc., so exercising an over-all control of the apparatus.

The synchronous motor driving the paper is fitted with a cam-operated contactor which generates pulses every $\frac{1}{10}$ min. The milking timer (Fig. 5) is operated by these pulses. It is essentially a pawl-driven drum calibrated in minutes and tenths with a full-scale reading of 15 min.

The driving and resetting operations are the same as for the recording apparatus. This will be seen from Fig. 5 in which *OC* is the operating coil, *RC* being the reset coil which lifts the ratchet from the driving drum. In the event of the timer reaching a full-scale reading before the cow finishes milking contacts (4) close so energizing the relay *R* which then connects the reset coil to the 24 V. circuit via contacts (1) and at the same time latches itself in by contacts (2). On returning to zero the inertia of the rotating drum causes the spring contacts (3) to be opened for a moment so dropping relay *R* out. The return to zero is sufficiently rapid to ensure that no pulse for the operating coil will arrive and cause the driving pawl to catch.

The timing mechanism gives a completely objective determination of the milk ejection time of the cow and so is a further step towards the ideal of the entirely controlled milking process required for a study of the conditioned reflex side of the physiology of milk ejection.

(*d*) *The mechanical milker*

The basic milking equipment is somewhat unorthodox and should be described in order to give a complete picture of the apparatus.

Fig. 6 gives the layout of the machine. An orthodox vacuum pump (*a*) supplies the

vacuum. The trap (*b*) collects any liquid carried over accidentally during the washing procedure. At (*c*) is an oil-damped weighted relief valve(4) which gives a precise control of vacuum. At the end of the milk pipe of the machine which is at floor level there is a tank (*d*) from the bottom of which the milk is pumped by the small single-acting rubber diaphragm pump (*e*). This pump runs at 40 r.p.m. and at the end of its shaft, which is driven by V-belt from the vacuum pump shaft, is a make-and-break which controls the

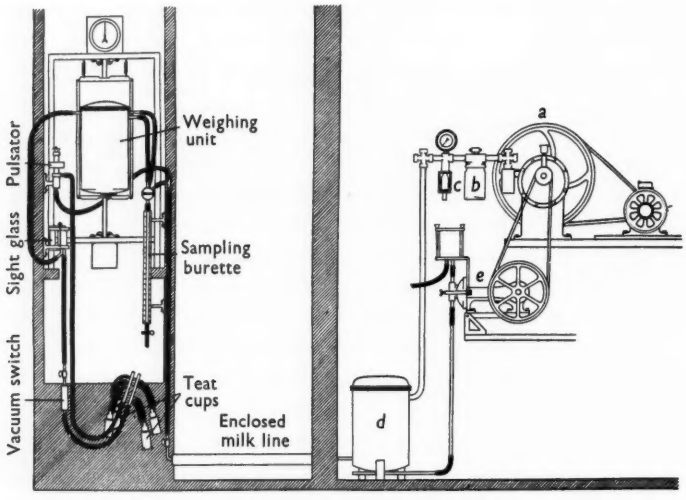

Fig. 6. Layout of mechanical milker.

magnetic pulsator valve. As will be evident from the figure, this machine eliminates all connexion between the separator room and the milking bails except the milk pipe at floor level and a wire connexion to the magnetic pulsator. The ratio of the pulsator is easily adjusted by altering the make-break ratio, and the pulsator drive current may be turned off and on simply by operating a switch. In other words, variations in the way in which the cow is stimulated by the pulsator can be made readily and rapidly—an essential for experiments on the effect of stimulation on the cow.

The pulsator valve is illustrated in Fig. 7. It consists of a brass tube (*a*) in which two sets of holes (*b*, *c*) are drilled. These act as ports, the lower set being surrounded by the annular connecting space (*d*) which communicates with the nipple (*e*) leading to the teat cups. The force to work the valve is supplied by the solenoid (*f*). The valve consists of a triple piston, the top member of which is made of magnetic metal while the lower parts are of brass. The bottom piston (*g*) moves above and below the ports (*c*), alternately connecting them to vacuum via the lower end of the valve cylinder (*h*) and to the air via holes (*i*) in piston (*j*) and the ports (*b*). Air is admitted to the teat cup pulsator chamber when the solenoid is energized, thus pulling the magnetic piston down, opening ports (*b*) and bringing piston (*g*) below the lower ports. The spring (*k*) pulls the valve back to the vacuum position thus closing the upper ports and raising piston (*g*) above the lower ports. The central member (*l*) connecting the pistons is a hollow tube which causes the same pressure to be maintained in the space above the valve (*m*) as in the vacuum system

connected at the lower end of the unit. This arrangement gives some damping to the movement of the valve. The middle piston serves to close the air ports so that high tolerances are not required in making a valve with low leakages. This enables the valve to have a loose fit and still function well.

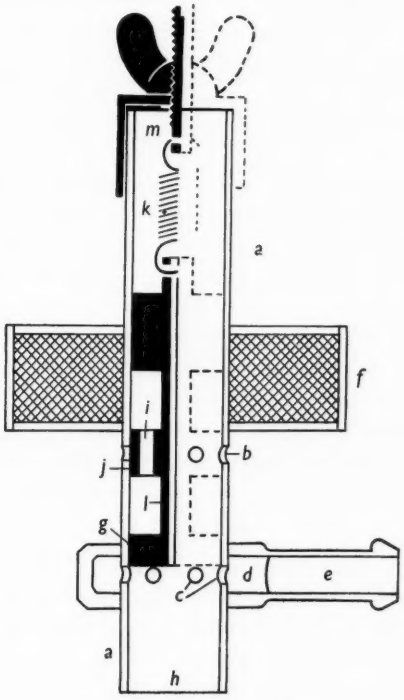

Fig. 7. Magnetic pulsator.

The system used in this pulsator valve has one main advantage over commercial types available so far. The current is on in the coil during the 'squeeze' phase which is only 20% of the total pulsator cycle. Hence, the power consumed is less than half of that required by a unit similar to commercially available valves working the opposite way round. The top cap is fitted with a rubber washer and an adjusting screw. To disassemble the valve all one need do is to lift off the cap so removing the complete valve assembly.

The apparatus described in the foregoing pages is complex and would, therefore, appear difficult to service. However, with the adoption of a completely pulse-operated system using orthodox aircraft and post and telegraph type relays with interchangeable contacts we have found the equipment to be very reliable. As a result of using the apparatus we have been able to obtain milk-ejection curves for every cow in our experimental herd at every milking without the need for an additional worker in the dairy. The measurement of milk weight and milking time may be done independently if it is desired to check the automatic instrument at any time. The device has now been in operation for over five years and has been found to be quite reliable. As experiments

demand, small modifications can be made from time to time in the wiring of the various control circuits, the whole circuit being quite flexible and readily altered.

The use of the automatic instrument has made possible the accumulation of a wealth of data on the milking characteristics of ordinary and of identical twin cows which would be almost impossible by any other method. A typical sample of milk-ejection curves drawn

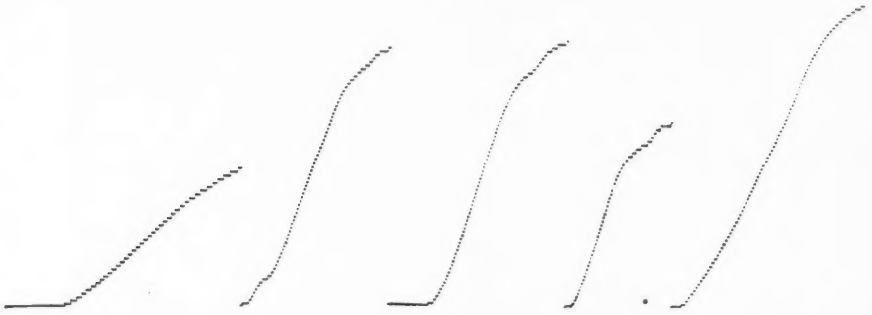

Fig. 8.

by the instrument is shown in Fig. 8. The records are clear, accurate and permanent, making possible the filing for future reference of the milking characteristics of all of our experimental cows.

SUMMARY

An improved milk-flow recording instrument is described which is completely controlled by the milking operations in an experimental dairy and which automatically indicates the end of milking in terms of a predetermined rate of milk flow. The instrument makes possible the recording of milk-ejection curves without changing the milking routine or requiring an additional operator in the milking shed.

The writers wish to acknowledge the assistance given by Mr D. J. Allen who constructed the mechanical components of the recording instrument and to Mr B. H. Millar who prepared the drawings accompanying this paper.

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637.223.5:537.311.3

523. A NOTE ON THE ELECTRICAL RESISTANCE AND THE KEEPING QUALITY OF BUTTER

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(With 4 Figures)

During the course of some earlier work (not yet published) on the physical properties and the quality of butter, the writer noticed that occasionally a sample was encountered which appeared to be very slightly rancid after only 3 weeks' storage in a cold store. These samples were observed to have a low electrical resistance, and to be of inferior texture, usually exhibiting some free moisture droplets when freshly cut. It appeared likely that there might be some definite relation between the resistance, the texture, and the keeping quality of butter. At the time of these experiments, however, the phenomenon only occurred with a few samples of one type of blended butter. The present note describes an experiment designed to explore the possibility that the relationship might be more general, and apply to other types of butter.

If butter were a perfect emulsion, with butter-fat as its continuous phase, its resistance would be of the same order as that of butter-fat, which is almost a perfect insulator as every water droplet would be enclosed by a continuous layer of fat, as is shown in Fig. 1 A.

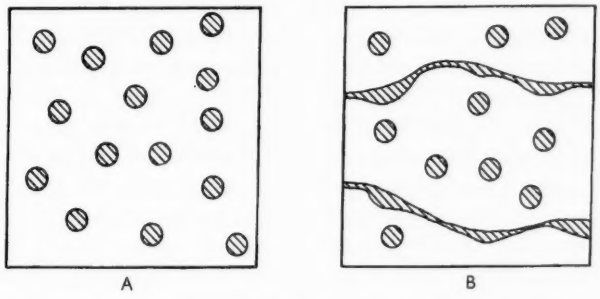

Fig. 1. Structure of butter (simplified). A, ideal; B, actual. Shaded areas represent water, plain areas fat.

In this case deterioration of the butter through bacterial development is limited (1). If the emulsion is not perfect there will be some continuous canals of water embedded in the fat, as well as the totally enclosed droplets (Fig. 1 B). Here there exist continuous conducting paths, so that the resistance of such a butter is considerably lower than that of butterfat, its value depending almost entirely upon the number and size of the conducting canals, and the concentration of salt in the water they contain. The number of bacteria in the canals may increase continuously, as there is a continuous supply of nutriment to them, and the butter will deteriorate. In an extreme case, the butter would not be an emulsion at all, but a simple mixture, and the resistance would be of the same

order as that of the appropriate concentration of brine. The specific resistance of butter may thus be expected to vary between an almost infinite value for a well-made emulsion, and about 30 Ω-cm. for a simple mixture of butterfat with 16% of water and 2½% of salt. In practice even a badly made butter does not approach this lower limit, as will be seen below.

For the present experiment, eight different samples of butter were used. Four of these were normal commercially blended butters, and the other four unblended butters. In order to increase the likelihood of differences between the blended butters they were obtained from different firms in widely scattered parts of the country. Two of the unblended butters were normal packeted butters, such as are available on the retail market, and these were obtained direct from the Ministry of Food stores. One of them was of a sweet cream type, and the other of a ripened cream type. The other two butters were specially made, again one from sweet cream, and one from ripened cream, but their water content was deliberately kept low, and the texture of these butters was very poor.

At the commencement of the experiment each sample of butter was examined independently by five members of a panel, all of whom had had considerable practice in examining butter. These judges were asked to comment critically upon the texture and the flavour of each sample. At this stage the comments on texture were principally concerned with the existence of three faults: the presence of free moisture droplets, the presence of air holes, and unevenness. It was found that the magnitude of these faults could be divided into four categories: 'none', 'slight', 'some' and 'much'; and in order to make the data roughly quantitative, these descriptions of magnitude were allotted scores 0 to 3 respectively, and the scores given by the individual judges added together. The principal comment on flavour was the degree of 'saltiness', and although this was of little interest in the light of the purpose of the experiment, for the sake of completeness it was allotted a score in a similar manner. Since the butters were quite fresh other comments were very few, and mainly indicated the personal preferences of the judges, rather than the existence of any faults in the butters.

At the same time, the distributions of free water droplets in the butters were examined by means of bromophenol blue indicator papers(2) pressed into contact with freshly cut surfaces of the samples. The water droplet distributions as observed by this method cannot be expressed in any precise quantitative form. The papers were examined, and the spots classified as small, medium and large, and the number of spots in each class was described as none, few, some or many. These divisions were quite arbitrary, but since there were large differences between the butters, they proved adequate to distinguish them. Typical examples are shown in Fig. 2.

In addition to this essentially qualitative examination of the samples, two electrical properties were measured; these were the dielectric constant, and the specific resistance. The same test cell was used for both these measurements. It consisted of two concentric cylinders whose radii were 1·575 and 2·135 cm., and was filled by taking a boring, gently, from the pat of butter, disturbing the structure as little as possible in the process. The dielectric constant was measured at an arbitrary fixed frequency of about 0·5 Mcyc./sec. The specific resistance was measured under two different sets of conditions. In both of them the measurement was made by means of a simple a.c. bridge, but in one case the power supply to the bridge circuit gave an output of about 6 V. r.m.s., at a frequency which could be varied between 150 and 1500 cyc./sec. The other power supply gave an

output of 250 V. r.m.s. at a frequency of 50 cyc./sec. The peak field strengths in the butter in the test cell, in these two cases, at the point when the bridge was balanced were then 15 V. and 630 V./cm. approximately; these will be referred to below as the low voltage and high voltage measurements respectively. No variation of resistance was observed with these samples as the frequency was varied between 150 and 1500 cyc./sec., and all the final low voltage measurements were made at 200 cyc./sec. The maximum specific resistance which could be measured with this apparatus was $2{\cdot}7 \times 10^8$ Ω-cm.

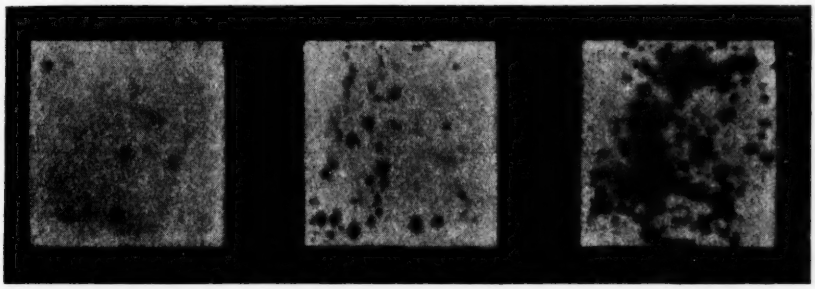

Fig. 2. Some typical moisture droplet distributions as shown by indicator papers. Left: butter C (blended); centre: butter E (sweet cream); right: butter H (sweet cream)

After the electrical measurements had been made on the sample, the butter was removed from the test cell and its total water content, and the salt content, determined by the standard methods. Thus all the quantitative physical and chemical measurements were made on the same sample of each butter.

The remainders of the butter pats were carefully wrapped in good quality parchment, and placed in a store, whose temperature was not controlled, but fluctuated between 70 and 80° F. The storage conditions were thus somewhat similar to the poor conditions which might be encountered in a domestic pantry during the middle of summer. At intervals the samples were removed from the store, examined by the panel of judges for flavour, and then replaced. After some 60 days all the samples had deteriorated considerably, especially in their texture, which had become very pasty in some cases. At this stage the judges expressed acute distaste for their task, and the experiment was terminated. An analysis of the comments of the judges showed that the principal remarks in addition to the saltiness observed at first could be classified under the two general headings 'staleness' and 'rancidity', and that the intensity of these flavours could again be described by the terms 'none', 'slight', 'some' or 'much'. Scores for these flavours were allotted in the manner described above.

The scores for the flavours were plotted against the number of days which had elapsed since the commencement of the experiment. These plots are shown in Fig. 3. In order to obtain an estimate of the date of onset of the undesirable flavours, and hence of the keeping quality of the butters, it was decided that a flavour should not be considered established unless it was given a score of at least three points, that is, unless at least a majority of the panel had reported it as 'slight', or one 'slight' and one more than slight', or unless one had reported it as 'much'. Actually only in one case was a score of exactly three points recorded for rancidity. In the other cases the dates of onset were estimated by interpolation between successive examination dates.

In Table 1 the results are summarized by ranking the butters in order for each quality judged subjectively, or in numerical order for each property measured. As the low-voltage resistance of the butters A, B, C and D was higher than $2{\cdot}7 \times 10^8$ Ω-cm., these could not be measured, and they have been allotted equal rank 1, although it is possible that differences would be found between them with a more refined technique. To distinguish

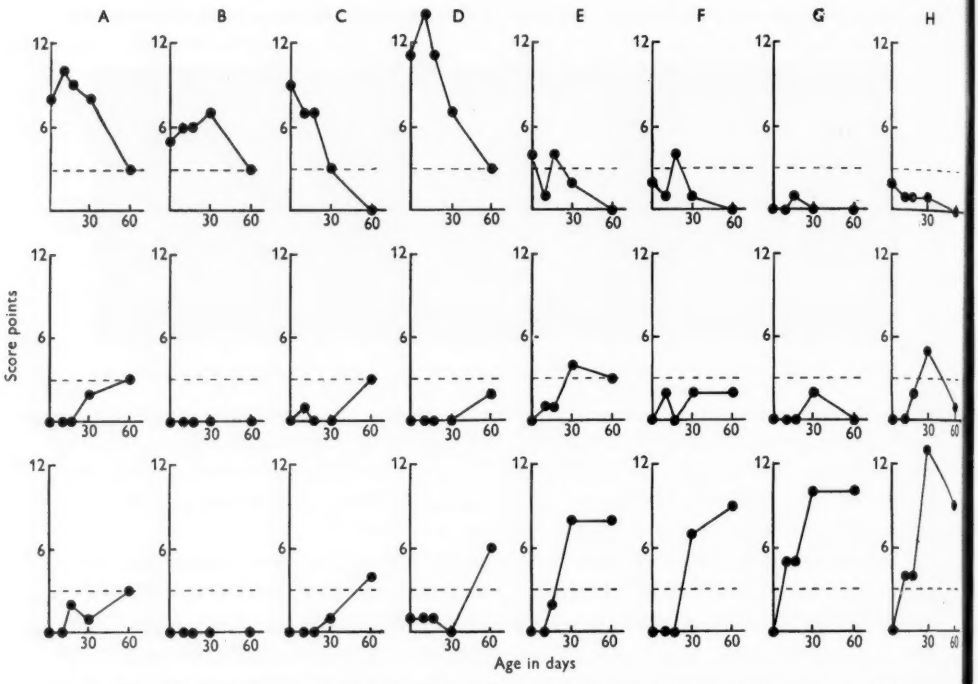

Fig. 3. Variation of flavour with age. Top line: saltiness; middle line: staleness; bottom line: rancidity. (Broken line = arbitrary threshold; see text.)

these from other cases of equal rank they have been entered in Table 1 in brackets. The onset of staleness, as defined above, was not reached with butters D, F and G, although some comments on this flavour fault were made. Accordingly, these have been allotted equal rank, but entered in brackets in the table. It is evident from an inspection of this table that the closest agreement in ranking occurs between the resistance measurements and the onset of rancidity. Both the low-voltage and the high-voltage measurements of resistance rank the poorest butters in the order in which they developed rancidity, the butter G, with the lowest resistance, developing rancidity first. The agreement between the high-voltage resistance ranking, and the keeping of the better butters is not so good. Butter B had the highest resistance, and had not developed any rancidity after 60 days, but butters A, C and D are not ranked in the same order in the two columns.

The relation between the measured (high-voltage) specific resistance and the date of onset of rancidity is shown graphically in Fig. 4A. A full line has been drawn through the lower points, where the ranking agreement was very good, and this has been continued as a broken line through the remainder of the points. In view of the arbitrary definition,

Table 1. *Ranking of butters by various properties*

Measurements

Table 1. *Ranking of butters by various properties*

Butter		Indicator papers Droplet distribution			Subjective assessments					Measurements				
Sample no.	Type	Small	Medium	Large	Free moisture	Air holes	Unevenness	Onset of rancidity	Onset of staleness	Water content	Salt content	Low-voltage resistance	High-voltage resistance	Dielectric constant
A	Blended	B	A	—	6	6	3	2	5	2	2	(1)	3	1
B	Blended	A	B	A	4	2	6	1	1	6	5	(1)	1	3
C	Blended	C	B	A	1	1	4	3	5	5	3	(1)	4	5
D	Blended	B	A	—	1	5	6	4	(2)	3	1	(1)	2	4
E	Sweet	C	B	B	4	2	6	6	7	4	7	6	6	6
F	Ripened	A	—	—	1	2	1	5	(2)	1	8	5	5	2
G	Ripened	A	B	C	7	8	2	8	(2)	7	6	8	8	7
H	Sweet	B	C	C	8	7	4	7	8	8	4	7	7	7

A = few; B = some; C = many.

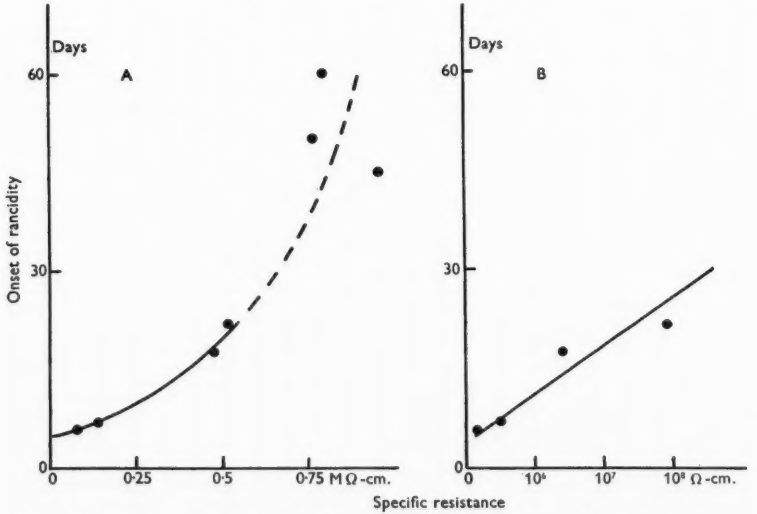

Fig. 4. Relation between keeping quality and specific resistance. A, high-voltage measurement; B, low-voltage measurement.

and the difficulties of determining the date, of onset of rancidity, the curve may be considered a good fit to the experimental points. The differences between the measured values of resistance at low voltage were much greater than those at high voltage, and the resistances of butters A, B, C and D, were all extremely high. Fig. 4B shows the date of onset of rancidity plotted against the logarithm of the specific resistance. The points referring to butters A, B, C and D could not be plotted as the specific resistance had a higher value than $2{\cdot}7 \times 10^8$ Ω-cm., and could not be measured.

In general there is fair agreement between the moisture droplet distribution determined by the indicator papers, and the texture assessed by the number and intensity of faults reported, and it may be observed that each of these, and particularly the droplet distribution, bears a fairly close relation to the total water content, that is, the higher the total water content (within the range 11–16%), the better the observed water droplet distribution, and the texture, as judged subjectively. This is in agreement with the findings in another experiment (not yet published). On the other hand, the keeping quality showed very little relation to these properties, except that the two butters G and H were the poorest in almost every way.

Apart from the relationships just mentioned there appeared to be very little correspondence between the different qualities and properties. It is particularly noticeable that the dielectric constant only bore a rough relation to the texture and droplet distribution, and none to the rate of onset of either staleness or rancidity. The decrease of saltiness with time (Fig. 3) is an interesting point. It is suggested that this is really a masking effect, due to the increase of undesirable flavours. Even butter B, although it did not develop any rancidity or stale flavour, had deteriorated considerably after 60 days, and had lost much of its original smooth firm body, so that it was less pleasant to the palate.

It would appear then, that the keeping quality is determined by finer structural faults in the butter than can be observed by means of indicator papers, or by the normal subjective assessment of texture. Only the grossest of differences can be picked out in this way. Measurements of electrical resistance may provide a more sensitive method of detecting these faults. The existence of minute conducting paths in the butter appears to be of much greater importance in inducing rancidity than the type of butter.

The experiment described above does not permit any more accurate deductions; further, more comprehensive, investigations into this problem are now being undertaken.

The author gratefully acknowledges the advice of Mr D. Sheppard on planning and interpreting the subjective assessments.

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524. THE INFLUENCE OF FEEDING TOCOPHEROL TO DAIRY COWS ON THE YIELD OF MILK AND MILK-FAT AND ON THE TOCOPHEROL CONTENT AND KEEPING QUALITY OF THE BUTTER

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The literature contains a series of reports on the influence of vitamin E on milk production and on the keeping quality of butter. It has been stated (6) that supplementation of the ordinary feed with 1 g. mixed tocopherols per cow per day resulted in an increase in the percentage of fat in the milk and an increase in the yield of '4% fat-corrected milk'. Other authors (5) found no such changes.

The feeding of a supposedly vitamin E-free basal diet was found by Burr, Lundberg & Chipault (1) to have an unfavourable effect on the stability of butter-fat in an accelerated oxidation test. With certain winter rations resulting in low resistance to oxidative deterioration of the milk fat, Krukovsky & Loosli (7) found that supplementation with 1–2 g. mixed tocopherols raised the tocopherol content of the milk-fat and increased its resistance to oxidative deterioration. However, no such improvement occurred when the tocopherol was fed to cows at the end of the winter season. The authors concluded that tocopherol supplements cannot be depended upon to maintain the tocopherol content of milk fat and to prevent oxidized flavours under all conditions.

Swartling (12) reported that incorporation of α-tocopherol in butter (2·5–10 mg./100 g.) had a pro-oxidative effect and tended to produce fishy flavours.

The amount of tocopherol in butter was determined by Reinart (10) at various times of the year, and in three different places in Sweden. Maximum values (about 2–3 mg./100 g. butter) were found in August or in September and October, times of the year when oxidative flavour occurs most frequently.

In order to study the influence of adding tocopherol supplements to typical Danish rations fed in winter at the Experimental Farms of the National Research Institute of Animal Husbandry, Copenhagen, the following investigations were made.

EXPERIMENTAL

Two series of experiments were carried out, one in the winter 1950–1, the other in the beginning of 1952.

Series 1 (1950–1)

The three periods of this experiment were as follows:
Preliminary period (no supplement): 27 November–7 January (42 days).
Experimental period (tocopherol acetate): 8 January–11 March (63 days).
Final period (no supplement): 12 March–15 April (35 days).

Two groups, A_1 and B_1, each consisting of nine cows, were used. Both groups were fed according to the plan indicated in Table 1. The cows in group A_1 continued on the unsupplemented ration throughout the experiment, whereas those in group B_1 received in the experimental period a daily supplement of 2 g. α-tocopherol acetate each. The tocopherol acetate (2 g.) was mixed with wheat flour (18 g.) and given together with the concentrate mixture of the feed. At the end of the experimental period one cow in group A_1 got a slight attack of mastitis. This cow and a corresponding one in group B_1 were left out in calculating the results. At the beginning of the experiment the average time interval from calving was 124 days in group A_1 and 113 days in group B_1.

Table 1. *Amounts of feed consumed per cow per day during the experimental period* (*Series* 1)

	Group A_1 (kg.)	Group B_1 (kg.)
'D-mixture'*	1·61	1·62
'A-mixture'†	1·84	1·74
Swedes‡	22·5	22·5
Sugar beet‡	18·9	18·7
Alfalfa silage‡	7·8	7·8
Alfalfa hay	3·0	3·0
Straw	2·0	2·0
α-tocopherol-flour (containing 10% α-tocopherol acetate)	—	0·02
Total Scandinavian feed units	11·65	11·55
Production feed units per kg. 4% fat-corrected milk	0·48	0·48
g. digestible protein per kg. 4% fat-corrected milk	64	64

* Cotton seed cake 25%, peanut cake 12%, soybean meal 9%, coconut cake 8%, sunflower seed cake 10%, palm cake 2%, rape seed meal 1%, peanut meal 1%, sunflower seed meal 5%, sesame cake 1%, linseed meal 10%, meat-and-bone meal 12%, liquid molasses 2%, mineral salts 2%.

† Cotton seed cake 6%, soybean meal 4%, coconut cake 4%, sunflower seed cake 4%, palm cake 1%, peanut meal 1%, sunflower seed meal 2%, sesame cake 1%, linseed meal 6%, bran-molasses 26%, rye bran 25%, barley 20%.

‡ The swedes and sugar beet were fresh and well washed; the silage was of good quality.

Table 2. *The yields per day per cow in the three periods of Series* 1 *and of Series* 2

	Preliminary period (no supplement)				Experimental period (supplements to groups B_1 and B_2, only)				Final period (no supplement)			
	Milk (kg.)	Fat (%)	Fat (g.)	4% milk (kg.)	Milk (kg.)	Fat (%)	Fat (g.)	4% milk (kg.)	Milk (kg.)	Fat (%)	Fat (g.)	4% milk (kg.)
					Series 1							
Group A_1	16·9	4·25	720	17·6	14·4	4·28	615	15·0	12·5	4·39	548	13·2
Group B_1	17·0	4·16	707	17·4	14·3	4·29	614	14·9	12·8	4·45	570	13·7
					Series 2							
Group A_2	17·8	4·39	779	18·8	17·1	4·44	757	18·2	15·9	4·44	707	17·0
Group B_2	21·5	4·12	883	21·8	19·9	4·12	821	20·3	18·0	4·16	750	18·5

The average yields per cow in the three periods are presented in Table 2.

The yield in the experimental period was practically the same for both groups, and the slightly greater increase in the percentage of fat in group B_1, as compared with group A_1, was not significant. It was concluded, therefore, that the tocopherol supplement had no influence on the yield of milk or on its fat content.

Samples of milk from the two groups of cows were delivered to the Government

Research Dairy, and immediately after its arrival it was heated to 60° C. and then skimmed by a laboratory separator. The amount of cream obtained from the milk of each of the groups was approximately 15 l. It was pasteurized at 85–90° C. and then cooled to 9° C. After 2 hr. at this temperature the cream was warmed to 20° C. by water at 26° C. and 10% starter added. The time for ripening was about 18 hr.

The churning of the cream and the working of the butter took place in two experimental churns, without rollers, and made of stainless steel (total volume about 40 l.). The butter was produced as washed butter and with a salt content of 1%. The pH of the serum of the butter was about 4·6, and the iodine value of the butter-fat about 27. Butter was made 4 times in the preliminary period, 21 times in the experimental period and 6 times in the final period. From the butter made in this way two samples of about 2·5 kg. were taken from each churning; they were kept in paraffined control tubs lined with parchment. The samples taken were subjected to the following examinations: (1) quality grading, (2) chemical analysis, and (3) bacteriological examination.

The quality gradings were made at the Government Butter Control Station after about 14 and 28 days of storage at 13° C. and after 3 months at −12° C. In grading the butter the classification was made by giving 0–15 points. Butter obtaining 10 points and more was considered faultless, whereas butter below 10 points was more or less defective. Before grading the cold-storage butter, it was taken from cold storage and stored for 4–5 days at 13° C. The following scale was used to express the degree of oily (oxidized) flavour: 0 = no oily flavour, 1 = slight oily flavour, 2 = oily flavour, 3 = strong oily flavour, 4 = very strong oily flavour.

The chemical examination of the butter comprised determination of its contents of salt and water, pH and peroxide value (ml. 0·002 N-$Na_2S_2O_3$ per g. fat (11)).

The bacteriological quality was assessed by plate count. The medium used for testing butter after storage for 5 days at 20° C. was a lactose-peptone-malt agar (pH 5·2). For butter stored for 5 days at 30° C. a sugar-free peptone agar (pH 7·2–7·5) was used. The bacteriological examinations showed that the bacteriological quality of the butter was satisfactory, and that in this respect the two groups of butter did not differ significantly.

The grading results are summarized in Table 3, which shows the average figures after 14 days' storage at 13° C. and after 3 months at −12° C. The quality gradings and the peroxide values show that although there was no appreciable difference in the quality of the butter in the preliminary and final periods, the butter from group B_1 in the experimental period was oxidized to a higher degree than the butter from group A_1. It would appear, therefore, that the addition of tocopherol may have promoted the oxidation processes which cause oily flavour.

Series 2 (1952)

The three periods of this experiment were as follows:
Preliminary period (no supplement): 7 January–10 February (35 days).
Experimental period (tocopherol acetate): 11 February–16 March (35 days).
Final period (no supplement): 12 March–15 April (35 days).
After the final period the cows were let out on pasture.

The two groups (A_2 and B_2) consisted of seven cows each. In the experimental period the cows in group B_2 received 2 g. α-tocopherol acetate each in the same manner as in Series 1, while the cows in group A_2 served as unsupplemented controls.

Table 3. *The flavour scores and peroxide values of the butters obtained from Groups A_1 and B_1 in Series* 1

		A_1				B_1			
		After 14 days at 13° C.							
Period	No. of experiments	Points	Remarks	Points for oily flavour	Peroxide value	Points	Remarks	Points for oily flavour	Peroxide value
Preliminary	4	10·5	Good quality	—	0·0	10·2	Good	—	0·0
Experimental	21	10·1		—	0·1	9·3	Oily flavour	—	0·4
Final	6	10·5		—	0·1	10·2	Good	—	0·1
		After 3 months at −12° C.							
Preliminary	4	7·8	Oily flavour	2·1	0·6	7·8	Oily flavour	2·2	0·6
Experimental	21	8·1	Oily flavour	1·8	0·6	6·4	Oily flavour	3·1	0·9
Final	6	8·9	Oily flavour	0·8	0·3	8·9	Oily flavour	0·9	0·3

The feeding plan is indicated in Table 4.

Table 4. *Amounts of feed consumed per cow per day during the experimental period (Series* 2)

	Group	
	A_2 (kg.)	B_2 (kg.)
'C-mixture'*	1·14	1·14
'A-mixture'†	4·87	6·73
Swedes‡	52·9	52·9
Alfalfa hay‡	4·25	4·25
Clover-grass hay‡	4·25	4·25
α-tocopherol-flour (containing 10% α-tocopherol acetate)	—	0·02
Total Scandinavian feed units	12·22	13·77
Production feed units per kg. 4% fat-corrected milk	0·42	0·45
g. digestible protein per kg. 4% fat-corrected milk	53	56

* Cotton seed cake 35%, soybean meal 33%, sunflower seed cake 5%, sunflower seed meal 5%, coconut cake 9%, rapeseed meal 7%, liquid molasses 4%, mineral salts 2%.
† Rapeseed meal 22%, rye bran 10%, bran molasses 34%, barley 17%, oats 17%.
‡ The swedes and the two types of hay were best quality.

At the beginning of the experiment the time interval from calving was 104 days in group A_2 and 90 days in group B_2.

From Table 2 it is seen that the α-tocopherol feeding did not influence the fat content of the milk. The yield in group B_2 was somewhat higher than that in group A_2, but since this was so also in the preliminary period, it is not likely that the difference was connected in any way with the feeding of tocopherol.

The tests for the keeping quality of the butter were limited to storage at −12° C. for 3 months. The results are summarized in Table 5. It is evident that the flavour gradings

Table 5. *Quality gradings of butter after* 3 *months at* −12° C. (*Series* 2)

		A_2				B_2			
Period	No. of exps.	Points	Remarks	Points for oily flavour	Peroxide value	Points	Remarks	Points for oily flavour	Peroxide value
Preliminary	4	9·4	Oily flavour	0·4	—	9·3	Oily flavour	0·4	—
Experimental	10	7·9	Oily flavour	1·6	0·6	7·6	Oily flavour	1·8	0·8
Final	6	8·0	Oily flavour	0·7	0·7	8·0	Oily flavour	0·9	0·7

did not show any noticeable difference between the keeping quality of the butters from the two groups, and even with the peroxide values the result for the B_2 butter during the experimental period was only very slightly higher than that for the butter of A_2. If this slight difference was significant, it would suggest that the addition of tocopherol under these particular feeding conditions again decreased slightly the keeping quality of the butter with respect to oxidation.

The tocopherol, carotene and vitamin A contents of the butters

The amount of total tocopherol in the butter was determined by a modified Emmerie-Engel method (3), using hot saponification in an atmosphere of nitrogen and *p*-hydroxyphenylglycine as antioxidant instead of the cold saponification previously used. In a few instances vitamin A and $\alpha+\beta$-carotenes were also determined, using chromatography on dicalcium phosphate and reading of the absorption at 325 mμ for vitamin A and at 450 mμ for $\alpha+\beta$-carotene.

Table 6. *Total tocopherol, and occasional determinations of vitamin A and α- and β-carotene, in butters from Series* 2 (μg/g.)

	Production date of butter (1952)	Group A_2 (control)			Group B_2 (supplemented)		
		Total tocopherol	Vitamin A	$\alpha+\beta$ carotene	Total tocopherol	Vitamin A	$\alpha+\beta$ carotene
Preliminary period (no supplement)	25 Jan.	11	—	—	12	—	—
	26 Jan.	12	—	—	—	—	—
	1 Feb.	11	—	—	11	—	—
Experimental period (tocopherol acetate)	15 Feb.	12	—	—	16	—	—
	16 Feb.	14	—	—	16	—	—
	22 Feb.	12	—	—	22	—	—
	23 Feb.	13	—	—	22	—	—
	1 Mar.	8	—	—	23	—	—
	7 Mar.	11	—	—	26	—	—
	8 Mar.	10	4·50	2·9	24	4·65	2·2
	15 Mar.	11	4·82	2·3	26	4·80	2·1
Final period (no supplement)	21 Mar.	—	—	—	21	—	—
	22 Mar.	10	—	—	22	—	—
	29 Mar.	12	—	—	12	—	—
	4 Apr.	11	—	—	14	—	—
	5 Apr.	—	—	—	14	—	—
On pasture	30 May	24	8·70	6·1	21	7·50	5·1
	6 June	23	8·85	5·4	20	6·60	4·5
	13 June	21	8·14	5·9	19	6·75	5·1

The results are presented in Table 6. It can be seen that the tocopherol content of the butter increased as a result of the tocopherol supplementation to about twice the amount found in the butter from the unsupplemented group. During the final period the tocopherol content decreased so that after 2 weeks the value was about the same as in the preliminary period. In the unsupplemented group the tocopherol value remained constant during all three periods. After the cows were let out on pasture the tocopherol content of the butter increased in both groups to about the level obtained by the supplementation. This value was about the same as that reported by Reinart (10) for summer butter in Sweden.

The few determinations which were made of vitamin A and carotene seemed to show that tocopherol supplementation did not result in an increase in the concentration of these two constituents of the butter.

The fact that the increased concentration of tocopherol in the butter did not result

in an improvement in its keeping quality suggests that in the unsupplemented group the proportion of tocopherol to autoxidizable fatty acids was already within the optimal range for preventing autoxidation of the butter during storage. The slight reduction in keeping quality resulting from tocopherol supplementation is in agreement with the pro-oxidative effect of mechanically incorporated tocopherol found by Swartling (12).

The fatty acid composition of the butter was not investigated, but from the results of other investigators (e.g. Mattson (8, 9)), it might be expected that the amounts of easily autoxidizable fatty acids, especially linolenic and a C_{18} conjugated acid, were relatively low at the time when the experiment was carried out. It is possible that an increase of the ratio of tocopherol to easily autoxidizable fatty acids beyond a certain limit may result in a more or less pronounced pro-oxidative effect. This might be the explanation of the results regarding the keeping quality. In summer or early autumn, when the butter has a higher content of easily oxidizable fatty acids and the keeping qualities are poorer, the tocopherol content is also higher. It is not known whether a still higher tocopherol content would improve the keeping quality in summer, but it seems unlikely.

A few trials have been made to assess the physiological value of the tocopherol content of the butter. For this purpose a species was chosen which is particularly sensitive to an insufficient proportion of tocopherol to autoxidizable fatty acids, viz. the chick. Two groups of ten chicks each were reared on a standard encephalomalacia-producing diet (2) in which the lard (30 % of the diet) was replaced by the same amount of butter-fat obtained by gentle melting of the butter and filtering. The butter-fat from the unsupplemented group of cows (A_2) contained between 9·6 and 13·2 μg. total tocopherol per g. while that from the supplemented group of cows (B_2) had 27·8–31·2 μg./g. throughout the experiment (35 days). Neither of the two types of butter-fat produced encephalomalacia, but white striation of the breast muscles appeared in one out of ten of the chicks receiving the butter-fat from the supplemented group. In the group receiving butter-fat from the unsupplemented group five out of ten had white striation of the breast muscles. This result suggests that the butter-fat contained enough tocopherol to check a tendency to encephalomalacia caused by the highly unsaturated fatty acids in the butter-fat, but that the amount of tocopherol, especially in the butter-fat from the unsupplemented groups, was not sufficient to prevent white striation of the breast muscles (4).

SUMMARY

Supplementation of a typical Danish winter ration with 2 g. of DL-α-tocopherol acetate per cow per day did not result in an increased yield of milk or milk-fat. The keeping quality of the butter was practically uninfluenced or possibly slightly reduced by the supplementation. The supplementation caused a rise in the tocopherol content of the butter from 10 to 12 μg./g. in the unsupplemented group to 23–26 μg./g. in the supplemented group. After the experimental period the amount of total tocopherol in the butter decreased rapidly. It increased again when the cows went to pasture.

Butter-fat obtained from the unsupplemented group with 9·6–13·2 μg. tocopherol/g. and from the supplemented group with 27·3–31·2 μg. tocopherol/g. did not produce encephalomalacia in chicks when incorporated at a level of 30 % instead of lard in a suitable test diet, but the amount of tocopherol present, especially in the butter-fat from the unsupplemented group, was not sufficient to prevent white striation of breast muscles in chicks under the same conditions.

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937.125.1

525. THE DETERIORATION OF MILKING RUBBERS

III. THE EFFECT OF FARM TREATMENT

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(With 9 Figures)

The previous papers in this series(1,2) described work which was undertaken to determine the cause of the quite rapid deterioration of natural rubber teat cup liners in use. In Part I it was shown that although rubber-consuming bacteria are present on the surface of liners in use, these do not make any appreciable contribution to the deterioration of the rubber. It was shown in Part II that the main reason for deterioration is the softening of the rubber, due to the physical effect of the fat continuously absorbed during the working life of the liner. Superimposed upon this effect is the attack of the rubber by oxygen, which under normal conditions is very slow. The oxidative breakdown is, however, considerably accelerated by the swelling of the rubber, and it was considered that this also contributed to the loss in stiffness experienced by the rubber.

The major part of the work described in the present paper was undertaken to determine the rate of accumulation of fat in natural rubber teat-cup liners and the rate of loss of stiffness of the barrel portion during service on the farm. Experiments have also been conducted in the laboratory which throw further light on the manner in which fat is absorbed into the rubber and the mechanism of the breakdown of the rubber. The role of other agents in assisting in the deterioration of the rubber has been investigated. The results of these experiments appear under the following headings:

I. Distribution of fat in used rubber teat-cup liners.
II. Accumulation of fat and its effect on the modulus of elasticity.
III. Loss of physical properties under farm conditions.
IV. Effect of stretch on the rate of fat absorption.
V. Structural breakdown of rubber in use.
VI. Effect of condition of milk on swelling.
VII. Effect of udder salves and washing agents.
VIII. Effect of atmospheric ozone.

I. DISTRIBUTION OF FAT IN USED RUBBER TEAT-CUP LINERS

Two natural-rubber teat-cup liners were used for the initial experiments. One was a moulded liner and the other a straight tube liner which had not been shortened, i.e. 'cut back', or reversed in the teat-cup during service. They were removed from the farm at the end of their useful life, cut into annular sections, each $\frac{1}{2}$ or 1 in. in length, and these were extracted with boiling acetone for 24 hr. The percentage volume extract was calculated from the following expression:

$$\frac{V_1 - V_2}{V_2} \times 100,$$

where V_1 = volume of section of used liner, V_2 = volume of section after extraction. The figure obtained does not represent the true amount of fat absorbed as the extraction is not quite complete after 24 hr. and also some of the constituents originally present in the rubber are extracted. The latter only amount to about 5% of the original volume of the rubber so that the figures given may be taken as close approximations to the true fat contents.

The results, which are given in diagrammatic form in Fig. 1*a*, *b*, indicate that fatty material accumulates mainly in the portion of the barrel in close contact with the teat and at the mouthpiece of the liner.

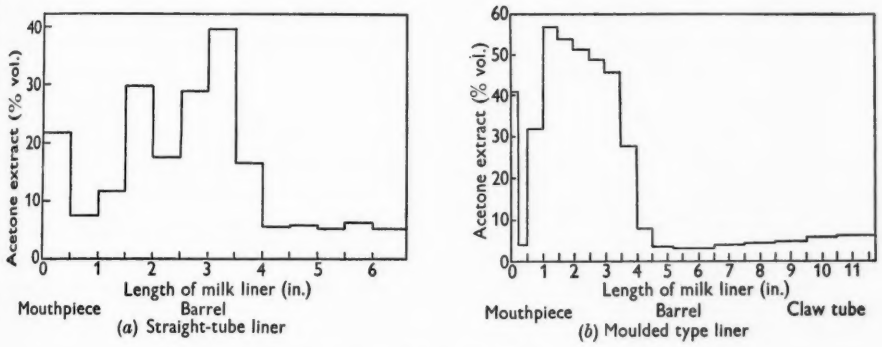


Fig. 1. Distribution of fat in liner after use on farm.

The tests were extended by slitting some used liners longitudinally, opening them out, cutting into small squares, approximately $\frac{1}{2} \times \frac{1}{2}$ in., and carrying out acetone extraction as before. The percentage volume extracts were calculated as before, and by grouping these results figures similar to contour maps have been constructed. Typical figures for three types of liner are shown in Fig. 2*a–c*. In the case of the moulded liners (Fig. 2*b*, *c*) the mouthpiece was not included in the test and the figures in these cases are representative of the straight barrel portion only. The 'height dimension' in these figures represents the percentage volume acetone extract. The subsidiary graphs shown on Fig. 2*a–c* were plotted using the totals of the columns and rows making up the contour map. The small sample squares were each allotted a contour number, i.e. according to the grouping mentioned above, and the addition of these numbers gives the points on the curves. These graphs are intended to show the variations in the average amount of absorption round the circumference of the liner and along its length. One of the observations made in the initial experiments using annular sections, namely that there is considerable fat absorption in the portion of the barrel in contact with the teat, is confirmed.

It is further shown that the maximum absorption in the barrel portion of the liner occurs mainly between the flex-lines. This is clearly shown in Fig. 2*a*, *b* and it has been confirmed by similar tests carried out on several other types of liner. The effect tends to be less noticeable with liners with large internal diameter, e.g. Fig. 2*c*, and this has also been confirmed by additional experiments. It may be noted that normally the flexing of a liner proceeds roughly in one plane throughout its life. Very occasionally more complicated flexing occurs in which three or more flex lines are distinguishable.

Two further observations may be made. First, the amount of fat absorbed in one half of a liner is often greater than in the other half. This can be seen in the graphs in the

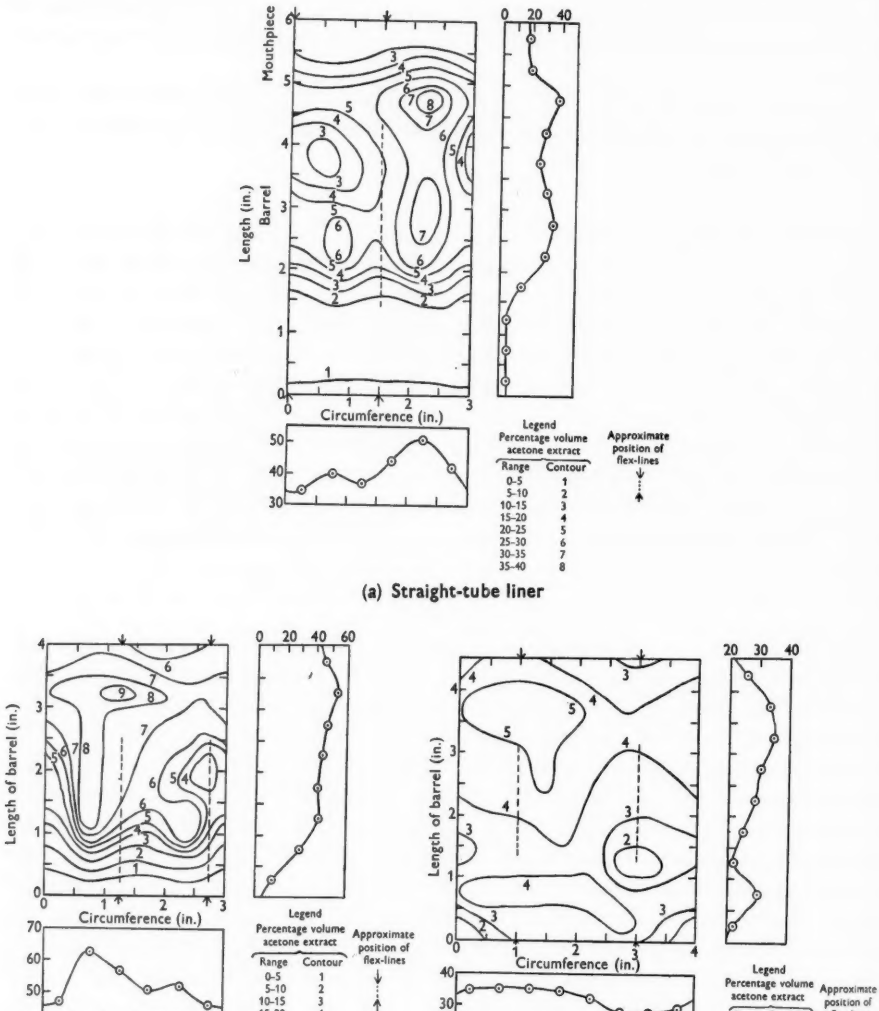


Fig. 2. Distribution of fat in liner after use on farm.

lower part of the figures which show average fat absorption round the circumference of the barrel. Secondly, an annular region of high fat content occurs near the end of the barrel adjacent to the mouthpiece.

Fig. 3 shows a photograph of the inside surface of a liner which had been used on a farm

and then stored for some time after removal from the teat cup. The positions of the flex lines are marked by arrows. The outline of the teat can be seen quite clearly due to blooming of the fat on the surface of the liner. This picture confirms the above observation that the maximum absorption takes place between the flex lines.

This phenomenon of blooming or diffusion of fat from the body of the rubber to the surface is dependent upon the stretch of the liner during use (see section IV). The solubility of fat in rubber increases with increase in stretch of the liner, and if sufficient fat is absorbed in the stretched state a supersaturated solution will be obtained when the rubber is relaxed. This gives rise to exudation of fat from the surface. Fig. 3 shows that the greatest exudation of fat and therefore the greatest absorption of fat takes place between the flex lines.

Fig. 3. Fat bloom on inside surface of used liner.

These results show that absorption occurs somewhat unevenly but in a definite pattern. The concentration of fat at the mouthpiece may be explained by the close contact of the rubber with the cow's teat and udder. Natural greases occurring on the skin and hair are absorbed by the rubber. This deterioration may be aggravated by the use of some udder salves (see section VII).

The origin and distribution of the fat in the barrel is more difficult to explain. It is suggested that there are four factors which have varying importance from farm to farm.

The first factor is the one mentioned above in regard to the attack of the mouthpiece. There may be traces of natural fats occurring on the teats. These will be absorbed by the rubber of the barrel as it comes in contact with the skin of the teats during milking. The annular region of high fat content near the end of the barrel adjacent to the mouthpiece is probably largely due to this type of attack.

The second factor is the absorption of butter-fat due to trapping of milk between the teat and the barrel as the cups are fitted to the cow. This occurs as a result of the normal milking routine. When the cups are hanging in an inverted position after milking a cow, a small amount of residual milk drains toward the mouthpiece and collects in the hood. When the cups are fitted on to another cow, the milk runs down the liner and is trapped between the teat and the rubber. It is suggested that then the emulsion breaks owing to mechanical agitation, which is caused by application and release of pressure during pulsation, and the fat is absorbed by the rubber. This would explain why the maximum fat absorption so often occurs between the flex lines in the barrel of the liner since there will be more effective trapping of the milk where the rubber and teat are in close contact.

The ease with which fat can be released from the emulsion was shown by a laboratory test. When a drop of milk was placed on a strip of rubber and agitated with a finger by rubbing and pressing to simulate the teat in the pulsating liner, it was quickly spread out and a sharp drop in friction occurred after 20–30 sec. Fat globules were then discernible on the surface of the rubber. It seems feasible, therefore, that a small amount of milk

trapped between the liner and the teat could be broken, and the fat released and absorbed by the rubber, within the time it takes to milk a cow.

A rough calculation of the amount of milk required to be trapped at each milking, so that the liner is rendered unfit for milking after a normal period of use, can be made. (In this paper the word 'milking' is used to signify the milking of an individual cow). If it is assumed that 3 ml. of fat are present in the barrel of a liner which has performed 3000 milkings, and that one drop of milk has a volume of 0·05 ml., then it would require 1500 drops of milk of 4% fat content to supply this fat. Therefore an average of only one-half of a drop of milk is required to be trapped and the fat released each time a cow is milked for the 3 ml. of fat to accumulate in the rubber.

The fat content will obviously affect the rate of swelling, and it may be noted that the fat content rises as milking proceeds (3,4). The fat content for the residual milk in the barrel of the liner may be nearer 10% rather than 4% as used in the above calculation.

The third factor is the absorption of butter-fat from milk trapped between the teat and liner during the actual milking process. The milk flow has been observed on a farm where straight tube liners are in use. A glass teat cup and a translucent liner were used for these observations. Early in the milking, milk was seen to flow smoothly from the teat when vacuum was applied to both sides of the liner. When during the pulsation cycle vacuum was applied only to the inside, the liner collapsed completely immediately below the teat. This resulted in cessation of milk flow. When the conditions were such that there was no air leak at the mouthpiece, as milking proceeded milk accumulated at the lower end of the liner and apparently did not flow smoothly away. After approximately 30 sec., during the portion of the pulsation cycle when the vacuum was applied to both sides of the liner, milk surged quite markedly from the lower end of the liner up around the lower end of the teat. The stream of milk from the teat could not be seen clearly during this stage of the milking and only when the milk flow had dropped towards the end of the milking, was it again easily observable. When the teat cup was removed, it could be seen that only the tip of the teat was wetted with milk. However, it seems likely that with types of liner having larger barrel diameter, milk may be trapped between teat and rubber as a result of the surging and frothing of the milk. It should be noted that if air was allowed to leak at the liner mouthpiece during the milking operation, the milk flowed rapidly away and there was no accumulation so that no surging round the teat was obtained.

The fourth factor is the possibility of milk residues souring or drying on the liner when stored between milkings (see section VI). Absorption of fat from this cause would vary greatly from farm to farm. On farms where a high standard of cleanliness is maintained, this effect will be of minor importance. An examination of Fig. 2*c* reveals an area of high fat content near the base of the barrel, which coincides with the portion of the rubber which is in contact with the sight glass used in this particular assembly. This would seem to be due to trapping of the milk between the glass and the rubber and subsequent absorption of fat due to drying or souring of the milk.

The pattern of fat absorption which has been found in a variety of used liners can be quite well accounted for by the above suggestions. One condition which is apparent in all the liners examined, i.e. the greater absorption in one half of the liner, is, however, not easy to explain. It may be connected in some way with the fact that normally when

the cups are fitted to the teats, the axis of the cup does not coincide with the axis of the teat, i.e. the teat takes up an oblique position in the cup.

The above considerations suggest that careful cleaning of the udder and teats before milking, and efficient rinsing of the cluster between milkings would assist in prolonging the life of the liner.

II. ACCUMULATION OF FAT AND ITS EFFECT ON THE MODULUS OF ELASTICITY

A test, in which groups of four straight tube liners were used for different periods of milking, was carried out on a farm where a three-unit bucket plant was in use. The liners were washed with cold water immediately after milking and sterilized daily with boiling water and hypochlorite solution. They were also removed weekly from the teat cups and scrubbed with a hot detergent solution followed by steam sterilization. Groups of four liners were fitted and one group was removed after 2 weeks use, the second after 4 weeks and so on. The group of liners, which was in use for 12 weeks had performed approximately 2000 milkings. A longer period of test was not possible as it would have been necessary to have shortened the liners and this was not desirable for this experiment.

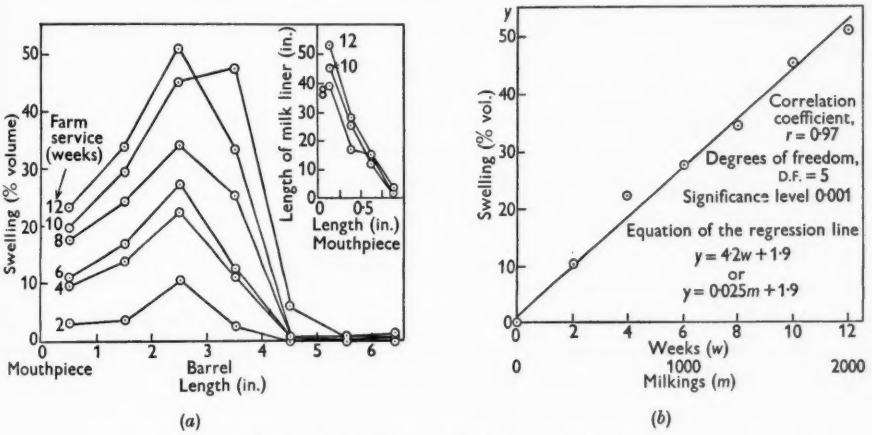


Fig. 4. Accumulation of fat in liner on farm.

After removal, tests on fat absorption and modulus of elasticity were carried out. One liner from each group was cut into annular sections and these were acetone-extracted for 24 hr. The percentage volume swelling of each section was calculated after correction for the volume of acetone-extract of similar sections of an unused liner. The results of these measurements are plotted in Fig. 4*a*. This figure shows the gradual swelling of the portion of barrel which is in contact with the teat. By considering the values for section 3 of each liner (for method of numbering see p. 349), Fig. 4*b* was constructed. The equation of the regression line was deduced and this is given on the figure. It can be seen that the fat absorption increases linearly with the number of milkings performed.

In the inset in Fig. 4*a* the percentage volume swelling figures for smaller divisions of section 1 of the liners removed after 8, 10, 12 weeks are plotted. In this case the normal annular section of 1 in. width was cut into four $\frac{1}{4}$ in. sections. It can be seen that the extreme edge of the mouthpiece is very badly affected by fat.

The percentage volume swelling at the edge of the mouthpiece and at the most swollen part of the barrel is seen to be about 50% after 12 weeks use. When the liner reaches this condition it no longer milks efficiently and, in actual practice, it is usual to reduce its length before this stage.

A second liner from each group on test was taken and the modulus of elasticity of five samples cut from it was measured under a stress of 5 kg./sq. cm. The swollen cross-sectional area when unstressed was used in all calculations of stress. Small dumb-bell-shaped samples were cut at points 1 in. apart at right angles to the length of the liner. They were marked 1–5 and number 1 was the sample cut nearest to the mouthpiece. By using a travelling microscope the measurement of the elongation was made on marks initially 1 in. apart on the narrow part of the dumb-bell sample. The results of these modulus tests are plotted in Fig. 5*a*. The softening effect of the fat is seen, and it is believed that loss in

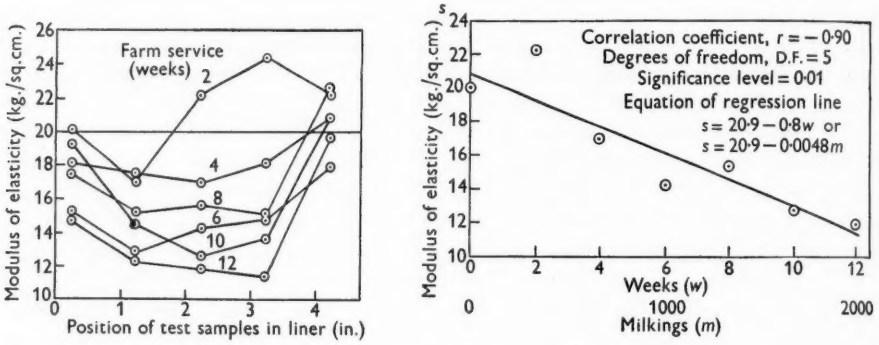


Fig. 5. Loss of stiffness of liner on farm.

milking efficiency is connected with this drop in modulus. In Fig. 5*b* a plot of the values of modulus for sample 3 is given. The existence of a linear relationship is confirmed by the correlation coefficient which has a value significantly greater than zero. The equation of the regression line is given on the figure.

The results for modulus are more scattered than those for fat absorption, but this is probably due to the existence of absorption patterns as described in section 1. The cutting of a dumb-bell sample may or may not coincide with the areas of maximum fat absorption.

Thus it is concluded that the rate of fat absorption is uniform during the life of a liner. This fat causes a gradual loss of stiffness (see section III), and a consequent deterioration of milking efficiency.

III. LOSS OF PHYSICAL PROPERTIES UNDER FARM CONDITIONS

The work described in this section was carried out on liners which had been removed from service because they were considered by the farmers to have finished their useful life. They were collected from several farms and their physical properties measured.

The method of measuring tensile strength was the standard one in which dumb-bell samples are used (5), and the modulus of elasticity measurements were made as in section II. Dumb-bell-shaped samples were cut parallel to the principal axis of the barrel, and three were obtained from each liner. The number of liners obtained from each farm varied but

the figures given in Table 1 are the mean values of at least six samples cut from a minimum of four liners.

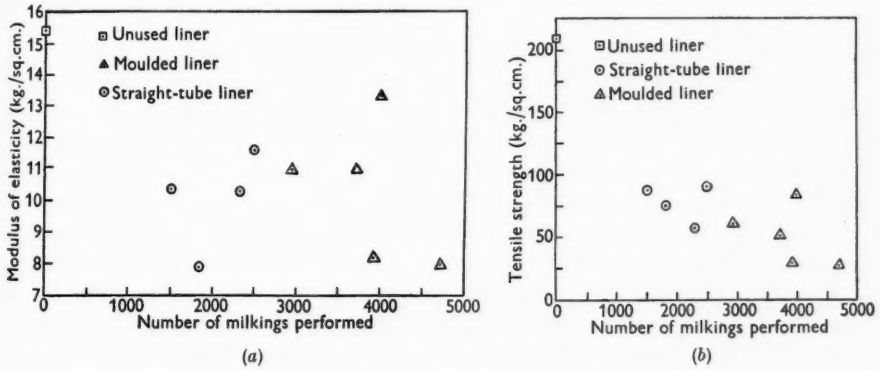
A variety of milker rubber compounds had been used on the several different farms. The tensile strengths and modulus values of these rubber compounds when new and unused were variable and the mean values together with standard deviations are included in Table 1.

Table 1

Farm	No. of milkings	Type of liner	Mean tensile strength (kg./sq. cm.)	Standard deviation	Modulus of elasticity (kg./sq. cm.)	Standard deviation
A	1500	Straight tube	88	12	10·3	0·9
B	1800	Straight tube	75	17	7·9	1·3
C	2300	Straight tube	57	23	10·3	1·3
D	2500	Straight tube	92	20	11·7	1·0
E	2900	Moulded	62	27	11·0	1·3
F	3700	Moulded	51	21	11·0	0·4
G	3900	Moulded	30	11	8·1	2·1
H	4000	Moulded	83	11	13·3	1·7
J	4700	Moulded	29	12	7·9	0·9
	0	Unused	210	20	15·4	1·3

The number of milkings performed by the liners on each farm is estimated to the nearest 100. It is calculated from the number of cows in milk and the period of farm service.

The farms all used different methods of washing and sterilizing the liners. On Farm D, the liners were shortened once during service. It may be noted that farm J does not maintain a very high standard of cleanliness and efficiency, whereas farms A and D are quite good.



(*a*) (*b*)

Fig. 6. (*a*) Tensile strength of used liners. (*b*) Stiffness of used liners.

The results are shown in Fig. 6*a*, where the mean tensile strength of the barrel portions of the discarded liners are plotted against the approximate number of milkings performed, and in Fig. 6*b* where the modulus values are plotted against the number of milkings. It can be seen that discarded liners vary markedly in their physical properties and that a set of liners rejected by one farmer may quite well be used by another farmer for a further period of weeks or months. One such case occurred when liners discarded by one farmer were used quite successfully by a second farmer for some considerable time. This is an

interesting point as it is intimately connected with the efficiency of milking and the standard of cleanliness maintained on a farm. It can be seen that farmer H has maintained his liners in better condition than farmer G, although both sets have performed approximately the same number of milkings.

IV. EFFECT OF STRETCH ON THE RATE OF FAT ABSORPTION

A short reference was made to the initial experiments carried out on this subject in Part II of this series (2). The results of a further experiment are given here.

The experiment was carried out with a standard milk liner compound moulded in sheets of 4 mm. thickness. Ring-shaped samples, 4 mm. wide, were cut from the sheets. These samples were tested in groups of three at constant elongation on a steel rack which was designed to stretch the samples up to 200% elongation at intervals of 25%. The

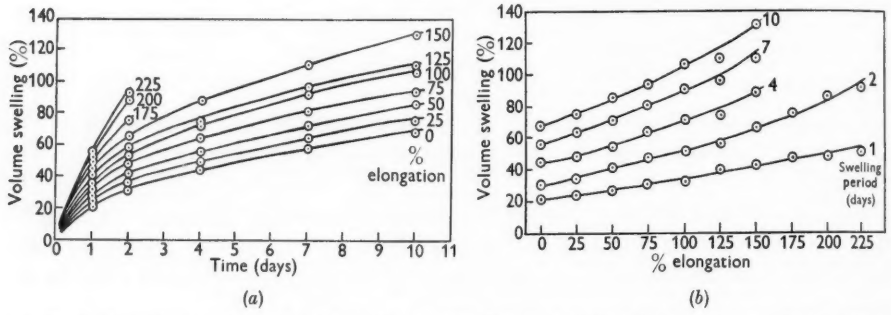


Fig. 7. Swelling of rubber at constant elongation. (*a*) Swelling against time; (*b*) swelling against elongation.

samples were stretched by means of two cylindrical steel bars inserted inside the ring and fitted into locating slots in the rack. The whole rack was immersed in lubricating oil contained in a bath fitted with a thermo-regulator, heating coil and stirrer. The bath was maintained at $35 \pm 2°$ C. The samples were weighed at intervals over a period of 10 days.

The results are plotted in Figs. 7*a*, *b*. The curves for 175, 200 and 225% elongation are incomplete because all the samples stretched to these elongations ruptured during the first 2 days. It can be seen in Fig. 7*a* that the actual swelling rate increases with increasing elongation. The swelling rates for all samples fall off with time; those under higher initial tension fall more quickly due to more rapid relaxation. After about 2 days the gradients of the swelling/time curves are approximately the same for all samples. In Fig. 7*b* the swelling is plotted against the initial percentage elongation. The curves show increase in swelling with increase in elongation. It was observed during these experiments that stretched samples which had become highly swollen, exuded oil when allowed to relax completely. This de-swelling was to be expected as such samples contained more oil than was necessary for saturation in the unstretched state.

These results are to be expected on theoretical grounds (6,7), and this relationship between stretch and swelling plays a part in the deterioration in practice. This has been shown by a test on two farms using straight-tube liners stretched initially to 30 and 50% elongations. The higher elongation was achieved by shortening the liners before fitting. As in the case of the laboratory experiment relaxation occurred. Eight liners were tested

at each elongation and were removed periodically for weighing to determine the fat up-take. The results given in Table 2 were not calculated on a percentage basis owing to the difference in original weight for the two groups. These figures show that a small, but definite, increase in rate of swelling was caused by an increase in stretch.

Table 2

	Increase in weight of group of eight liners (g.)		Period in use (days)
	30% elongation	50% elongation	
Farm 1	17·5	17·5	17
	36	37·5	38
	53	52·5	54
	59	61	70
	66	70·5	88
Farm 2	20·5	22	20
	36·5	39	38
	53	54	54
	59·5	63	72
	68	70·5	90

V. STRUCTURAL BREAKDOWN OF RUBBER IN USE

In the last section of Part II in this series (2), a laboratory investigation into the effect of absorbed fat on the deterioration of rubber was described. It was shown that the oxidation of rubber in the swollen state is very much more rapid than that of unswollen rubber. The oxidation causes reduction in the length of the molecular chain and hence a weakening of the vulcanized rubber which is manifest by reduction in tensile strength and stiffness. Confirmation that this occurs during farm use was obtained in the following manner.

Four straight-tube liners which had undergone several weeks' use on a farm employing daily heat sterilization, were used in this test. Each liner was cut into seven 1 in. annular sections. The liners appeared to have been reversed during use, and the sections from the ends which had acted as mouthpiece during the latter part of the working life of the liners were numbered 1. The other sections were numbered 2 to 7 in sequence. The sections were extracted to substantially constant weight in boiling acetone; they were weighed in air and water before and after extraction in order to determine the amount of fat absorbed. The latter was expressed as a volume percentage based on the volume of unused rubber. After extraction the acetone was allowed to evaporate and dumb-bell-shaped test samples were cut. The modulus of elasticity of the samples at an elongation of 500% was determined. The area of cross-section of the unstressed samples was used in the calculation of the stress. Table 3 gives the mean results for each section.

Table 3

Section	Percentage volume fat absorption	Modulus of elasticity at 500% elongation (kg./sq. cm.)
1	29·6	113
2	37·4	100
3	46·6	103
4	54·7	99
5	38·1	104
6	23·2	114
7	18·0	123

These results are given in diagrammatic form in Fig. 8. This shows quite clearly that breakdown of the rubber is related to the amount of fat absorbed by the rubber. One would expect this effect to show more markedly with liners which are regularly steam-sterilized, as the effect of heat is to increase considerably the rate of oxidation. In the case of liners which are not subjected to heat during use the extent of the oxidation may be small and their removal may be necessitated merely by loss of shape or tension due to the swelling concurrent with fat absorption. Generally speaking, liners deteriorate more quickly on farms using heat sterilization and the reason is probably to be found in the

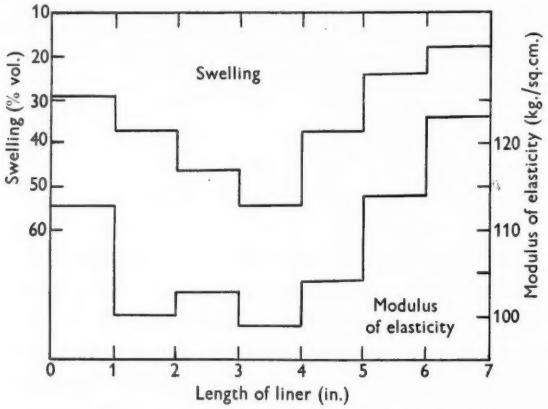


Fig. 8. Swelling and modulus of elasticity after extraction.

enhanced oxidation. There are, in fact, two effects occurring which reinforce each other. These are the acceleration of oxidation due to swelling and enhanced swelling due to oxidation. This latter effect has not been referred to before, but it is known that as the rubber network is broken down by oxidation, the rubber becomes more easily swollen. The combined action of these two factors probably causes oxidation to play an important part in the deterioration of rubber liners under some conditions of operation.

VI. EFFECT OF CONDITION OF MILK ON SWELLING

In Part II of this series (2) the very slow swelling of rubber in milk which was preserved during the test by addition of a small amount of mercuric chloride was mentioned. A test has been made using similar rubber swollen in milk to which no preservative had been added. Twelve samples, each $2 \times 1 \times 0{\cdot}06$ in., were weighed and immersed in pasteurized milk which was maintained at $35 \pm 2°$ C.

Water was added daily to maintain the original level in the tank. After 1 day the milk, which was stirred continuously, had turned yellow, and oil was detectable on the surface. A semi-solid mass formed on the surface after 2 days and this was removed at the end of 4 days after squeezing dry. By the end of 6 days oil was floating in large pools on the surface of the sour liquid.

In Fig. 9, the curve for percentage volume swelling against time is given. The approximate pH of the milk was obtained by the use of test papers and these values are given on the same figure. It can be seen that as the milk turned sour the rate of swelling of the

rubber increased rapidly. The swelling of 40 % after 6 days compares with 3 % in preserved milk under the same conditions.

The surface condition of the samples at the end of the test was interesting. Ridges covered the surface of each sample in a random fashion. The cross-section of a ridge was roughly hemispherical with a diameter of approximately 1 mm.

This type of attack by milk on a liner occurs rather infrequently and is due to neglect. Careless washing and leaving the liner in the teat cup for long periods (see section I, Fig. 2*c*) can lead to drying or souring of milk at the junction of rubber and metal or rubber and glass.

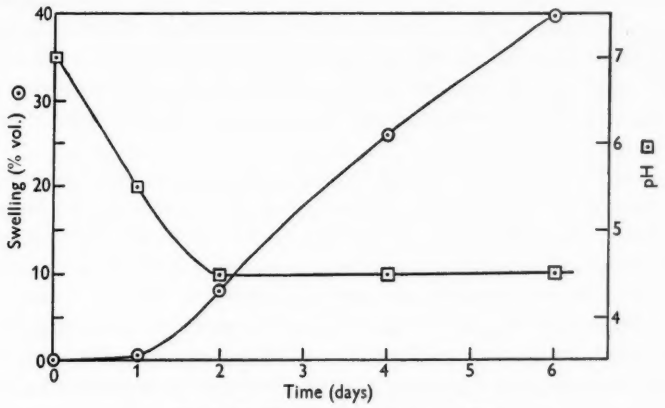


Fig. 9. Swelling of rubber in sour milk.

VII. Effect of udder salves and washing agents

Weighed samples of a vulcanized rubber liner compound, $2 \times 1 \times 0{\cdot}06$ in., were immersed in four proprietary udder salves heated at $35 \pm 2°$ C. This temperature was chosen as being approximately the body heat of the cow. After 7 days the samples were removed, cleaned, weighed and the percentage volume swelling was calculated.

Table 4

Sample	Percentage increase in volume
A	127
B	44
C	3
D	31

The results given in Table 4 show that the constitution of the udder salves on the market varies widely. Those based on greasy materials, such as lanolin, are absorbed rapidly into the rubber. Where frequent applications of the type A are used the barrel and orifice of the liners would suffer from being swollen by the salve as well as by fatty material from the cow's hair and skin. This is possibly the reason for the very rapid swelling of liners reported occasionally. The results for the type C show that there is at least one material in common use which is not harmful to rubber.

In order to determine whether the various detergents in use for cleaning rubber liners

have any deleterious effect upon the rubber, samples were immersed for 1 hr. in boiling 5% aqueous solutions of eight proprietary detergents and sterilizing agents, sodium carbonate and caustic soda. After the treatment the rubber samples were allowed to cool and the tensile strength and modulus of elasticity were determined. In every case the results were not significantly different from those obtained from rubber immersed in boiling water for the same time nor from those from untreated rubber. The concentrations used in this experiment were much greater and the temperature higher than those obtaining on the farm. This suggests that the cleaning and sterilizing agents in common use do not cause any appreciable deterioration of the rubber.

There are two possible ways in which these materials may, however, influence the deterioration of the liner. First, they may attack the surface of the rubber and render it susceptible to the trapping of milk residues and so to the formation of a layer of milkstone. When the latter is present the liner becomes more difficult to clean and under these conditions the rate of fat absorption may be increased. Further work on this point is required.

The second way in which it was thought that the detergent could influence the deterioration of the liner is in the efficiency of removal of fat. Obviously the more efficiently the fat is removed from the surface the less fat is actually absorbed into the body of the liner. It was considered also that the detergents might even assist in removing some fat from below the surface layers. In the previous paper(2) a test was described in which used liners immersed in 10% caustic soda for several weeks showed evidence of removal of fat. In order to find whether there is likely to be any removal of fat under the normal conditions of washing, the following test was devised. A number of $2 \times 1 \times 0{\cdot}06$ in. samples of a rubber compound were swollen in rape seed oil for 2 days at $35 \pm 2°$ C. During this time the rubber increased in volume by approximately 50%. The swollen rubbers were then boiled for 10 min. in 10% solutions of various proprietary materials, caustic soda and sodium carbonate and also in acetone. As was expected the amount of oil extracted by the aqueous solutions was very small, varying between 0 and 7% of the oil present in the rubber. In the case of the samples boiled in acetone, about 40% of the oil was removed. It is concluded that the fat removed from the liner, other than that removed from the surface, during normal washing is negligible. Naturally when the rubber was soaked over a long period in caustic soda, as mentioned above, sufficient time was available for the fat to diffuse from the body of the rubber to the surface. If it is desired to remove fat rapidly from rubber an organic solvent is required. This method is not, however, to be recommended for practical use as the solvent would remove, not only fat, but also antioxidants and other protective agents from the rubber.

VIII. EFFECT OF ATMOSPHERIC OZONE

A troublesome type of deterioration of rubber connecting tubes used in the air and milk systems is the cracking which develops where the tube is forced over the end of a metal connexion, and where the tubing is bent for long periods, e.g. in storage between milkings. In the former case the cracking is in the same direction as the longitudinal axis of the tube. In the latter case, if the bend always occurs in the same manner, it is found that the side of the tube which is under stretch cracks while the opposite side which is under compression due to the bending of the tube shows no tendency to crack. The cracking is due to ozone which attacks stretched natural rubber rapidly. The concentration of

ozone in the atmosphere at ground-level is of the order of 4 parts per 100,000,000, and even this extremely low concentration is sufficient to cause severe cracking. The worst cracks are obtained when the elongation is at a critical value which is in the neighbourhood of 20% according to Newton(8). This author states that at elongations both above and below this value less severe cracking is obtained. The circumferential stretch which is obtained when the rubber tubes are forced over the metal connexions on two milking machines in common use varies from 7 to 20%. Thus the stretch which occurs at these points is, in the majority of cases, in the range in which severe cracking is obtained. Also the stretch due to bending, although variable, is also in the elongation range where cracking is severe.

The method generally employed to combat this type of cracking is the addition of certain types of wax to the rubber compound. After vulcanization wax diffuses to the surface to form a very thin layer which protects the rubber from ozone attack quite effectively. If the film is cracked or damaged in some way the exposed rubber is susceptible to attack and because of this it is not possible to eliminate cracking completely in the case of articles such as tubing which may receive more or less rough treatment. Under most farm conditions, rubber properly compounded with wax is fairly free from severe cracking.

CONCLUSIONS AND SUMMARY

The accumulation of fat in a rubber liner occurs mainly in two regions, the mouthpiece and the portion of the barrel in contact with the teat. It is suggested that the mouthpiece is attacked by body fats and udder salves mainly, whereas the barrel absorbs milk-fats from milk trapped between the rubber and teat. The accumulation of fat increases linearly with the number of milkings performed.

The loss of milking efficiency is connected with the stiffness of the rubber compound, and this latter property decreases linearly with the number of milkings. The length of service of a liner on the farm is shown to be largely dependent on the individual farmer and his standards of efficiency and cleanliness.

The presence of fat in rubber leads to an enhanced rate of oxidation and consequent loss of physical properties. This has been confirmed in practice. An increase in the tension of a liner increases the rate of swelling and the souring of milk on rubber gives a similar result. The use of detergents has no direct deleterious effect on the physical properties of rubber. They are not effective in removing fat from below the surface layers. The occurrence of ozone-cracking of parts of milk liners or tubing, which are under tension, is discussed and the use of waxes in the compound as a remedy is described.

Some of the types of deterioration described above can be minimized by the maintenance of a high standard of cleanliness of the rubberware. Frequent removal of liners from the teat cups for thorough cleaning is essential.

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526. A STUDY OF VARIATION IN TWIN CATTLE

I. GENERAL DESCRIPTION

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The identification, collection and experimental use of one-egg cattle twins is now a well-established research procedure. In an informative review of this development Bonnier & Hansson(1) have referred to the fundamental work of Kronacher, his colleagues and successors, on identification, and to their own pioneer studies on their exploitation for genetical and nutritional studies. Since then, Hancock(2,3) has published a series of papers describing his work with twins in New Zealand.

In 1948, the Animal Breeding Research Organization began a search for one-egg twins in the mid-western counties of England, with the object of using them in its breeding research programme. As a foundation for future work, the first experiment was to consist of a uniformity trial in which the performance of one-egg twin heifers could be compared with that of two-egg twins and pairs of half-sibs. It would have the double function of indicating the statistical efficiency of the three types of pairs relative to each other and to pairs of unrelated animals, and of throwing some light, by virtue of the several degrees of genetic relationship involved, on the remarkably high heritabilities for numerous characters deduced from previous trials with one-egg twins in Sweden and New Zealand.

DESIGN OF EXPERIMENT

In broad outline, therefore, the plan was to collect fifteen pairs each of one-egg and two-egg twins, and half-sibs (ninety animals) which was as many as could be accommodated, and to rear and milk them all under the same conditions.

Within pairs of one-egg twins, the only known source of variation would be pre- and post-natal accidents of environment (e^2). In two-egg pairs this source would be supplemented by genetic variation which in a fair sample of pairs should approximate to half the genetic variance (g^2) characteristic of the populations from which their parents came. Half-sib pairs should show the greatest variance since they would contain three-quarters of the genetic variance and maternal effects (m^2) due to their having two different dams instead of one as with twins, as well as an error variance presumably of the same order of size as that shown by twins.

Influences due to farm of origin and season were to be excluded by (*a*) selecting half-sibs that were born at the same time on the same farm, and (*b*) buying pairs of two-egg twins and half-sibs immediately each pair of one-egg twins was obtained so that sets of three pairs would be formed comparable in season of birth.

Variance between sets, arising from a combination of all genetic and environmental factors, would be of limited interest. Since the collection of the calves would be continued over several months at least, farm, season and breed differences would be inseparably combined. Certainly there should be no attempt to estimate heritabilities from the ratios of the variances within pairs of one-egg twins to the variances between them.

It was appreciated that the partition of intra-pair variances into e^2, g^2, m^2 components might well be an over-simplification. No account is taken there of other factors which might contribute to intra-pair variance of two-egg twins and half-sibs, namely, deviations due to dominance and epistasis and genotype-environment interaction due to differing responses of the two members of these pairs to changes in environment common to both. Although the design of the experiment would not permit separate assessment of these additional factors, however, it was expected to show whether it was necessary to postulate them at all in order to explain the results.

In practice, the plan, although in the main realized, could not be followed exactly. Half-sib pairs proved very difficult to find. They had not been advertised for as had twins; they had to come from small farms using one bull to make reasonably certain that they were half-sibs, and they had to be born within a week of each other and healthy. In consequence, one-egg pairs of twins were not matched as exactly with half-sib pairs in time of birth as had been hoped. Another complication was caused by losses of pairs of each kind after the experiment had started. Some extra pairs had been carried to cover these losses, but they naturally failed to fit exactly into the places which became vacant. Dates of birth of the pairs which were in the experiment at three months of age were distributed somewhat irregularly over the 8 months from October 1948 during which they were collected. Each animal, soon after purchase, was tattooed in the ear with the letters MZ (for monozygotic) DZ (for dizygotic) or HZ (for half-sib), plus a serial number and an A or B to distinguish it from its mate. All were dehorned at about 3 weeks old.

Considering that most of the calves were bought at 4–7 days old from non-attested farms in winter, losses from all causes must be considered fairly low. One or both calves from five pairs died or were sold owing to scour (1); chronic unthriftiness (1); blackleg (1); reaction to tuberculin (2). The point is not unimportant because it has a bearing on the question of selection of pairs. Ideally, all the animals should have been drawn at random from the same population and, so far as is known, they were. Such a small sample, however, could only by a stretch of the imagination be regarded as representative of the whole cattle population of the three dairying counties covered in the search for twins. The population was and is in fact subdivided by the practice or neglect of attestation and pedigree registration and it is extremely mixed as regards breed. Friesian and Shorthorn blood predominates but Ayrshire and Hereford crosses are also common. One-egg twins may well occur at random, but it is doubtful whether those offered for sale were drawn proportionately from all sections of the population, and the same applies to the other types of pair. What can be said is that the pairs actually bought were with one exception non-pedigree and with one exception non-attested. Their approximate breeding is shown in Table 1, from which it may be gathered that even if non-representative the three major groups are similar as to the nature and variety of their breed content.

Table 1. *Breeding of twin and half-sib pairs*

	MZ	DZ	HZ
Friesian	2	2	1
Shorthorn	6	4	6
Friesian × Shorthorn crosses	3	7	5
Miscellaneous	5	2	2

Selection for health was exercised by the officer collecting twins, and he was instructed not to purchase pairs the members of which were markedly different in weight. All

healthy one-egg twins were bought regardless of type or breeding and none showed any marked intra-pair differences in weight at the time of purchase. Whether the restriction on weight affected his choice of two-egg and half-sib pairs it is impossible to say. Selection after purchase, that is culling, was carried out only for failure to pass the tuberculin test, save that heifers whose mates had died or reacted were also disposed of. Many of the calves scoured, some badly and with complications, but all were carried on.

MANAGEMENT

Reduction of uncontrolled environmental variance is much to be desired in most experiments and particularly in those employing twins. Unfortunately, it is still very uncertain how best to direct efforts to secure this reduction. Swedish workers have gone to great lengths to secure control over the quantity and quality of food intake. In New Zealand, where twins are kept entirely out of doors, food intake is under a very different sort of control. Neither of these methods was appropriate to our conditions. Buildings available were unsuited to exact methods of feeding, and continuous indoor or outdoor feeding were equally impracticable. Moreover, it was hoped that this experiment would supply data on the use of twins to other institutions in Britain similarly placed, that is without facilities for refined techniques. The husbandry regime was therefore designed to give as much control over environmental variables as possible without major departures from normal farm routine. Details of it will be given where appropriate in later papers, but the main features may be listed as follows:

(*a*) Rearing on the bucket with whole milk; weaning at 10 weeks.
(*b*) Rationed concentrates and silage during winter; hay unrationed.
(*c*) Grazing in summer with 2 lb. concentrate supplement daily during the first summer.
(*d*) Natural service at 15 months for calving at 2 years old.
(*e*) Steaming-up ration fed.
(*f*) Liberal concentrate ration after calving to cover milk production and growth.
(*g*) Partial randomizing of positions in the dairy byre.

Levels of nutrition throughout were intentionally higher than normal. This was partly due to the wish to have the heifers well enough grown to be served at 15 months. The main reason, however, was the decision to deal with the problem of achieving equal treatment by conducting the experiment under conditions approaching equal opportunity. Bonnier, Hansson & Skjervold (4) have stated an equality principle as a guide to the achievement of equal treatment for animals. On one interpretation the animals are fed according to a common fixed scale varying with, for instance, weight or yield of milk. On another they are all fed the same quantities over a fixed period of time. On both, the assumption of equality of treatment seems to involve equating need to some function of age, or weight or milk production. Whatever the merits of this assumption, it was impossible to control intake of all food, hence the approach to the problem of feeding the twins in the present experiment differed from that reported from Sweden.

In so far as the heifers were housed together, grazed together and given the same kinds of food when indoors they may be said to have been treated alike. Food intake, however, was uncontrolled in summer when they were at grass and only partially controlled in winter. Hay was always available but silage and concentrates were fed according to body weight and (after calving) to milk production. Quantities of silage fed in winter were

rather small (maximum 15 lb. per day) so that it would rarely be necessary to weigh back uneaten residues, but concentrates were offered on a liberal scale and small amounts were often left uneaten after 1 hr. On the whole, therefore, it seems fair to say that there was very little restriction on intake, and that the animals had equal opportunity to express themselves fully in terms of growth and milk production in so far as the various foodstuffs were qualitatively adequate. With a motley collection of calves, this treatment was expected to develop differences in appetite and performance, and was practicable. The only major deviations from ordinary farm routine were the weighing of individual rations of silage and concentrates and the tying up at feed times. The heifers, until shortly before calving, were penned in groups of six to eight except when at grass.

Live weights were recorded every 2 weeks and used in winter (October–April) to determine the rations for the succeeding 2 weeks. Each heifer was considered individually. When a decline in live weight was observed, the rations were left unchanged. After each weighing in winter the animals were moved to another pen, but pairs remained together.

At 15 months of age the heifers were put to one or other of a pair of one-egg twin bulls. Services were completed in October 1950, and in November those first served were due to calve. Whereas the calves were collected over a period of 8 months, services and calvings extended over a period of 9 months.

Until November 1950 the heifers were kept at the Organization's dairy farm in Staffordshire. In that month, however, it was necessary to transfer them all to new quarters on another farm in Peeblesshire. The heifers, many of which were advanced in pregnancy, had thus to face a road journey of 240 miles, adapt themselves to a different type of housing, and become accustomed to new personnel. In the ensuing few months there were four abortions, two premature and eight still-born calves, several difficult calvings, and a long record of grazed hocks and other minor ailments. Although the transfer may well have caused much of this trouble, it seemed advisable, after half the heifers had calved, to reduce the concentrate rations. From February onwards heifers due to calve were given the rations appropriate to their weight but the extra steaming-up concentrates were withheld. For this or other reasons the calving record was thereafter much improved.

The pairs had been split when they were introduced to the new byre in the process of stalling the heifers in order of calving. This brought about a partial randomization which was thought desirable on theoretical grounds. In the following spring the heifers went out to grass but not all at once. An effort was made to arrange that the two members of each pair went out to grass after equal intervals since calving. This worked well enough except that the last few heifers not allowed out became so restless that it was deemed advisable to abandon the attempt to achieve this kind of uniformity for them.

The heifers were again put to the bull 3 months after calving save that some flexibility was allowed with pairs which had calved rather far apart in order to try to bring them back into step.

Further information about many of the matters mentioned above will be given when detailed results are presented.

TWIN DIAGNOSIS

Although the pairs were classified as one-egg, two-egg and half-sib, there is no certainty that every pair was placed in its correct class. Methods of diagnosing one-egg twins were basically the same as those used in Sweden and New Zealand, but it is by no means

improbable that observers have somewhat different standards of judgement so that whilst agreeing unanimously in respect of most pairs they would disagree on occasion. In the absence of critical objective methods of diagnosis, all that can be said of the one-egg twins in this experiment is that they were classified at about 1 week old and that only one pair later turned out to be of two-egg origin. The possibility remains that other pairs were also dizygotic although they developed no differences judged to be significant.

The same sort of uncertainty can exist with respect to two-egg twins although there is less reason to accept it. They are plentiful and can easily be found with known genetic differences in pattern or colour. One of the two-egg pairs, however, in this experiment might possibly have been one-egg, since even after calving differences between the two heifers were very slight. This type of error is made more likely if any pair not accepted as one-egg is classed as two-egg and if for one reason or another it is convenient to include them in the experiment. With two-egg twins there is also the faint possibility that they are by two different bulls. For half-sibs this risk is somewhat more serious. Since market prices were offered for such calves, nothing was to be gained by representing them as half-sibs when they were not, and the only doubt about their relationship arises from the chance that unknown to the breeder two sires were in fact involved. This possibility was reduced by taking half-sib pairs from one-sire herds.

It is an interesting question in experiments of this sort how far reclassification of pairs is permissible during the course of an experiment. When an experiment begins with calves one or two weeks old, it is convenient to class pairs as one-egg or two-egg shortly after birth and without the advantage of being able to compare their late-developing characters. Although it may seem desirable on occasion to change the classification, a revision, apart from its effect on the design of an experiment, cannot be lightly made.

Much can be said for it if environmental effects, whether intended or not, can be excluded as causes of the dissimilarities or lack of them on which it is based; for an experiment is obviously impaired if the presence or absence of genetic effects is wrongly assumed. There are, however, at least two arguments against revision. The first is the difficulty of being sure that environmental effects accumulating since birth do not affect judgement. Late developing characters which might be guides to the identity or otherwise of genotypes are those, like bony conformation or udder type, which are supposed to show relatively little non-genetic variation; but not enough seems to be known of the heritabilities of such characters to justify much assurance in using them in preference to those available in young twin calves which have not had so long to acquire characters resulting from chance differences in environment. Much seems to depend on subjective assessment of just what differences are likely to be of genetic origin. The second argument is that it is improper to vary the classification as a result of knowledge which it was the purpose of the classification to obtain. It would be wrong in an experiment such as this, one object of which was to compare the butter-fat percentage of members of pairs of one-egg twins, to exclude one pair because it was thought to show too large a difference.

Until objective and critical methods of twin diagnosis are available, it is necessary to be satisfied with subjective diagnosis and accept the possibility of occasional error. As will be shown, the subjective method, even if not infallible, is very effective when practised on calves 1 week old. Mistakes in diagnosis seem unlikely to exceed and are probably fewer than one in ten and it seems reasonable to suppose, without condoning needless inaccuracy, that errors of this order of magnitude will not matter much except

in crucial experiments involving very few pairs of twins. For these circumstances, specially selected pairs of irreproachable likeness could be used.

In accordance with these views only one diagnosis has been revised—a pair originally classed as one-egg was transferred to the two-egg group when evidence of differing conformations became noticeable at the yearling stage. The change affected the analysis of the data (most of which was subsequently obtained) but not the animals themselves.

DATA

Since the experiment lent itself to the analysis of any character, efforts were made within the limits of staff, techniques and time, to record observations on as wide a variety of traits as possible. These include growth, milk and butter-fat production, body dimensions, fertility and teeth. Analyses of the data obtained will be presented in later papers.

SUMMARY

A general preliminary description is given of a uniformity trial with twin cattle, the object of which was to study sources of variation within pairs by comparing one-egg and two-egg twins with pairs of contemporary half-sibs. Problems relating to equality of treatment and accuracy of diagnosis are discussed.

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527. A STUDY OF VARIATION IN TWIN CATTLE

II. FERTILITY

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Results of research on the contribution made by heredity to normal variation in fertility in dairy cattle have been conflicting. Abnormal variation in the sense of sterility or marked reduction in fertility has been reasonably attributed to special genetic situations due either to single gene effects or to inbreeding degeneration; but unsystematic and comparatively minor fluctuations have sometimes appeared to show a genetic component and sometimes have not (for references see Young (17)). Where it appears, however, this indefinable genetic influence has been small compared with the variation arising from non-genetic causes. This is perhaps to be expected of a highly complicated and sensitive process subject to interruption at many stages and considered of necessity in many studies as an 'all-or-none' trait.

Observations made on numerous characters of one-egg twins analysed by conventional methods, have regularly led to estimates of the genetic component of variance which appear inflated when compared with those derived from breeding herds of singly-born cattle. It is of interest, therefore, to know whether the evidence of hereditary control of fertility appears relatively strongly in twin cattle reared together. Being exactly contemporary, they are presumably exposed at the same time to whatever environmental agents influence fertility, and for that reason could be expected to behave more similarly than non-contemporaries. This expectation would apply to both one-egg and two-egg twins, but the latter could show a greater diversity within pairs in so far as their genetic dissimilarity is capable of producing it. An experiment of this type, expanded to include pairs of half-sibs, has been described in general terms in a previous paper (8).

The purpose of this account is to present an analysis of the following aspects of fertility: age at first heat, intervals between heats, services per conception, gestation period, sex and twinning in the progeny.

The experiment began with fifteen to seventeen pairs of each kind, acquired as 4- to 7-day old calves over a period of 8 months. Their first heats consequently occurred over an extended period lasting from August 1949 to June 1950. Since calves born in January 1949 or earlier were at grass in the following summer, while those born later did not go out to grass until the summer of 1950, first heats were observed both indoors and out. Heifers which had been at grass, however, were put out daily during the winter whenever the weather and soil permitted. In November 1949, that is, after the oldest heifers had been seen on heat, a vasectomized bull was obtained, and he was available to assist in the detection of heat thereafter. The method of using him was to let him run with the heifers whenever they were at grass, and to expose heifers, kept indoors, to him daily. Sometimes his enthusiasm outran his discretion. The decision to record a heat was therefore not based entirely on his judgement nor on the raddle marks he left, but on a combination of criteria. During the relevant period all heifers were seen at close quarters

daily when tied up to receive their concentrate ration (this applies even to those at grass which received 2 lb. concentrates daily during their first summer) and were examined for signs of heat: i.e. swelling of the vulva and a clear discharge followed by a blood-stained discharge 2–3 days later. Additionally the usual behaviour pattern of heifers on heat, mounting and submission to mounting, was noted and supplemented by closer inspection and exposure to the bull. The heifers were always close at hand, and thus during the daytime they were under more or less continuous supervision. For convenience, monozygotic, dizygotic and half-sib pairs are referred to as MZ, DZ and HZ respectively.

Age at first observed heat

Exact data on age at puberty in domestic cattle are scanty (11). Bentley & Phillips (3) give the average ages at first oestrus of thirty Holstein heifers in Wisconsin as 7·44 to 9·56 months for five variously fed groups. Wille (16) stated that in North German cattle breeds a regular oestrous cycle is first established at 12–15 months of age. In Japan, Masuda, Onishi & Kudo (13) found that forty-one Friesian heifers reached sexual maturity at 9·6 months and at a live weight of 239·4 kg. In South Africa, twenty-eight grade Sussex cattle with some Africander blood averaged about 17·6 months old at first heat. Other cattle differently treated averaged about 14·3 months (14). Some three-quarters of the Holstein and one-quarter of the Zebu heifers studied by Duckworth (9) in Trinidad were equally late maturing, with an average age at first heat of 17 months.

The average ages at first observed heat of the twins under discussion turned out to be $10\frac{1}{2}$–$11\frac{1}{2}$ months; in detail monozygotic (MZ), 334 days; dizygotic (DZ), 351 days; and half-sibs (HZ), 317 days. These numbers may be too large if some first heats were not observed; they may be too small if some records were made in error. For six MZ, nine DZ and nine HZ heifers which were recorded as on heat before the bull was used, the average ages were 316, 320 and 317 days. This might mean either that the oldest MZ and DZ heifers were earlier maturing, or that before the bull was used signs of heat were accepted by the recorder more readily than after. The former interpretation is preferred in the light of details about growth rates which follow. Furthermore, the bull is assumed to have aided and not hindered the onset and discovery of heat periods.

Table 1. *Analysis of variance in age (days) at first heat*

No. of pairs	Mean square within pairs	Mean square between pairs	F	P
MZ 16	386·47	3908·25	10·11	< 0·1%
DZ 14	657·14	2420·96	3·68	< 5%
HZ 14	646·61	835·58	1·29	> 20%

An analysis of variance is summarized in Table 1. MZ heifers as a class showed relatively wide variation, but any one heifer tended to behave as her twin did. The ratio of variances within and between sets of DZ heifers was large enough to justify thinking that DZ twins had a tendency to behave similarly, although not so markedly so as MZ twins. No such tendency was evident among HZ heifers considered as an isolated group. Owing to the diversity in time of birth, breed and origin of the pairs, between-pair variances are probably less reliable and certainly more difficult to interpret than within-set variances. The ratio of these in MZ, DZ, HZ order is about 1:1·7:1·7 (P about 20% for a ratio of 1:1·7 with 15 D.F.).

When age at first heat was plotted against live weight at 12 months of age, the slower growing animals were seen to have come later to puberty. The correlation between the weight of an animal and its age at first heat proved to be: MZ, $-0{\cdot}60$ ($P<0{\cdot}1\%$); DZ, $-0{\cdot}62$ ($P<0{\cdot}1\%$); HZ, $-0{\cdot}13$ ($P>20\%$).

The low figure for HZ heifers was due to the fact that there were no really slow-growing pairs among them. In all three classes, heifers reaching 525–550 lb. or more by the time they were a year old showed no relation between growth rate and age at first heat. A curvilinear regression would fit the facts, but the number of slow-growing pairs hardly justifies the effort of calculating it. Asdell(2) has referred to the late onset of heat in underfed calves at Cornell.

When allowance is made for the co-variance of growth rate and age at first heat (assuming a linear regression) the data given in Table 1 are modified to the form given in Table 2. The correction reduces the between-pair variances considerably, and alters the

Table 2. *Variance in age at first heat corrected for growth rate*

	Mean square within pairs	Mean square between pairs	*F*	*P*
MZ	331·02	2435·22	7·36	< 0·1%
DZ	674·30	1245·10	1·85	<20%
HZ	677·77	826·88	1·22	>20%

ratio of within-pair variances to about 1:2:2. Although the MZ pairs as expected show a greater degree of concordance than the DZ and HZ pairs, the observed ratio could well be of chance origin. The probability of a 1:2 ratio with 15 and 13 degrees of freedom is about 10%.

Had the MZ pairs been unaccompanied by DZ and HZ pairs it would have been easy to attach overmuch importance to the fact that pairs differed markedly while individuals within a pair did not. If the three intra-pair variances are accepted as the most reliable evidence available, it will have to be concluded that the case for a degree of genetic variation is not proven.

Table 3. *Percentage distribution of oestrous cycle lengths*

Length in days	Chapman & Casida(5)	Present data
4–16	4·9	8·4
17–27	60·3	71·8
28–33	4·9	3·7
34–44	29·9	9·5
44+		6·7

Intervals between heats

Intervals between heats up to the time of first conception ranged from 7 to 135 days with a well-defined principal mode at 18–22 days and a minor but recognizable one at 38–42 days. A frequency distribution of oestrous cycle lengths according to a scale used by Chapman & Casida(5) is given in Table 3. The two sets of figures are not strictly comparable because Chapman & Casida based theirs on 690 cycles from a breeding herd of Holstein cows over a 10-year period, whereas the present data consist of 571 cycles from unserved heifers recorded over 15 months. Both sets agree however that many apparent cycle lengths are far from normal even when 'normal' is defined as generously as 17–27 days. Some idea of the frequency of silent or very short heats that are not observed

may perhaps be obtained from the size of the second mode. Of the cycles recorded here most (say 8%) may have followed a heat which was missed. This would raise the frequency of 'normal' cycles of 17–24 days in our material to about 80%, but of these every tenth may be missed even when much trouble is taken to detect it. Since persistently short or long intervals would tend to obliterate the second mode it seemed unlikely that such intervals could be demonstrated. An analysis was however made to see whether any sign of individuality could be found. For the purpose, intervals longer than 30 days were excluded, and consecutive short intervals that were obviously portions of an 18–22-day cycle, such as 8 and 12 days, were combined. The result was a mean square between heifers (87 D.F.) of 8·52 and a mean square within heifers (366 D.F.) of 6·92 which does not suggest that heifers can be distinguished from one another on this basis. The mean lengths of interval (counting long intervals as two or more cycles) were MZ, 20·1; DZ, 20·7; and HZ, 20·3 days; with standard deviations (within animals) of 3·48, 2·83 and 2·84 days respectively. Analysis of variance revealed no significant differences either between pairs, or between types of twin.

A contrary result from nine pairs of one-egg twins of mixed grade breeding has been reported from Minnesota by Dziuk, Hervey, Brundage & Donker (10). They found significant differences between pairs on the basis of which heritability of length of oestrous cycle was estimated at 61%. Since their conventions for deciding what was and what was not to be regarded as a normal cycle were not the same as those used in this study, the point of interest which seems to emerge is how to define and recognize the normal oestrous cycle.

No association of long intervals with season and management factors could be found. Red, red roan, and white animals seemed to show less sign of heat than black ones, but this did not appreciably affect the average intervals—reds 25·9, blacks 24·2 days—estimated from individual averages based on unadjusted records. Long intervals due to failure of heifers to show heat, or to failure to observe heats were more frequent immediately after the first observed heat than later. For the first three intervals the unadjusted averages were: MZ, 38·0, 24·4, 23·8 days; DZ, 26·9, 22·8, 23·8 days; HZ, 26·2, 25·1, 21·2 days. Although it is possible that, with the passage of time and growing knowledge that a heifer is due, heats may be more efficiently detected, the impression remains that young heifers are more difficult to 'catch' on heat, no matter whether they are indoors or out. Experience with these cattle is in keeping with the idea that first oestrus, attainment of ability to conceive, and full sexual maturity are successive stages in the reproductive history of a cow (1, 11).

Services required for conception

As they reached 15 months of age, heifers were inseminated naturally by a pair of one-egg twin bulls. On the criterion of calves born, bull A settled heifers 43 times from 68 services (63%) and bull B 44 times from 67 services (66%). One heifer was settled by one artificial insemination after five failures by natural services included in the bulls' records. All heifers were got in calf. Average number of services per conception were MZ, 1·4; DZ, 1·3; HZ, 1·9, the difference (MZ+DZ) *v.* HZ being highly significant. The single-born relatively heavy HZ heifers were more difficult to settle under the conditions imposed than were the twins.

Although numbers of services required is not a normally distributed variable, the data

may be analysed in the usual fashion (6). Results are shown in Table 4. Neither MZ nor DZ pairs showed any sign of distinctive behaviour. HZ heifers also showed as much variation within pairs as between pairs, but for some reason they were altogether more variable than the twins. Those which required three or more services were exceptional in neither growth rate nor age at first observed heat.

Table 4. *Analysis of variance of services per conception*

	No. of pairs	Mean square within pairs	Mean square between pairs
MZ	16	9·50	6·22
DZ	14	4·00	3·70
HZ	14	25·00	20·86

Some of the cattle were retained for second and third pregnancies. The necessary services were provided by (*a*) one of the original bulls, (*b*) another pair of one-egg twin bulls, and (*c*) a non-twin Aberdeen Angus bull. On the necessarily small subgroups, conception rates by bulls could hardly be expected to show significant differences. Of 363 services given in 3 years by five bulls, 248 (68·3%) resulted in pregnancies (MZ, 69·6%; DZ, 71·7%; HZ, 64·0%). Since animals were culled from the herd at various times after their first lactations, these figures are based on a summation from year to year of results from a diminishing herd. The numbers of pairs surviving to the third pregnancy were MZ, 12; DZ, 11; and HZ, 10. Culling was never based on failure to conceive. For the third pregnancies, not all of which have been completed, evidence of conception has been based when necessary on pregnancy diagnosis, or on clinical symptoms in conjunction with absence of oestrous periods. Several abortions have occurred but all proved negative for *Brucellus abortus*.

One HZ cow and one MZ cow, failing to settle after three services for their second pregnancies, received hormone treatment and conceived at the next service. The HZ cow subsequently had twins. These were the only veterinary episodes directly relevant to questions of reproduction.

To determine whether differences in service record (not apparent before first pregnancies) became evident with time, an analysis of services per conception (bulls ignored) has been made on complete pairs surviving to their third pregnancies. This is given in Table 5. None of the three long-term effects considered (permanent differences between pairs, or between mates, and successive pregnancies) seem to have been important save perhaps differences between MZ pairs.

Table 5. *Analysis of variance of numbers of services per conception on twin and half-sib pairs surviving to third pregnancy*

	MZ		DZ		HZ	
Source	D.F.	Mean square	D.F.	Mean square	D.F.	Mean square
Between pairs	11	1·186	10	0·433	9	0·748
Within pairs	12	0·625	11	0·394	10	0·567
Pregnancies	2	1·500	2	0·561	2	0·850
Residue	46	0·572	42	0·497	38	0·582

Following segregation of the components of variance, repeatabilities of numbers of services per conception have been estimated as follows: MZ, 0·142; DZ, −0·035; HZ, 0·107.

All are small and non-significant, as would be expected from the evidence already led that the cattle have shown little sign of individuality in respect of their services records to date.

To test effects of season on conception rate for first pregnancies, successful services were divided into two classes: winter (October to March) and summer (April to September). Those accomplished in winter turned out to have been preceded by relatively fewer unsuccessful services than those in summer but, on a χ^2 test, not significantly so. Their slight superiority stemmed from the fact that those HZ heifers whose turn it was to be served in summer conceived less readily than the older winter-served HZ heifers which differed little if at all from the MZ and DZ heifers at any time. This narrows the source of the initial HZ inferiority but does not explain it. The heifers responsible were all retained for third pregnancies, and it can be said that they were subsequently prompt to settle. Once again, therefore, conception seems to have depended rather heavily on circumstances at or near the time of service.

Heat and service after conception is judged to have occurred five times. These services have been ignored. In all cases full-sized apparently full-term calves were born after pregnancies of 256 days or less as calculated from date of last service. This frequency (5·5%) is in accord with previous experience (7).

Gestation period

During their first pregnancies, four heifers aborted after 3–6 months' pregnancy for some reason other than *Br. abortus*. These were excluded from the ensuing analysis of gestation period along with two MZ heifers which produced underweight calves after gestation periods of 252 and 264 days. The mean lengths of period were similar to those reported earlier (1).

For first pregnancies, the principal results of analysis were as shown in Table 6. There is evidence that something tended to cause pairs of animals to behave alike, for the differences between pairs are clearly significant for MZ and DZ twins. Whether or not this something was genetic, does not emerge from the data. Certainly the mean square within MZ pairs was only 6·81, whereas that within HZ pairs was 11·29, but this ratio had a P of about 20%. A later batch of eight MZ pairs, also treated uniformly, had a mean square of 37·8 (which can be reduced to 14·6 by the exclusion of one pair).

Table 6. *Analysis of variation in length of first pregnancies*

	No. of pairs	Mean square within pairs	Mean square between pairs	F	P
MZ	12	6·81	18·78	2·76	< 5%
DZ	11	11·08	36·39	3·28	< 5%
HZ	11	11·29	17·65	1·56	> 20%

With the aid of data on second pregnancies the analysis has been carried further but regrettably on two fewer complete pairs. Since the figures add little to Table 6 that cannot be briefly stated, the details will not be given. Differences between sets became rather more marked, but those between first and second pregnancies were significant only in HZ pairs. Persistent differences between members of a pair were also significant only in HZ pairs. After extracting these effects the largest residual variance was in the DZ group and the least in the HZ group (either with or without the contribution from

non-significant effects due to pair-year interaction). Only the differences between pairs can be regarded as established and because of the correlation between one calving date and the next, the difficulty of attributing them unequivocally to heredity remains. That there is genetic variation in gestation period, however, can hardly be doubted in the light of published data (see, for instance, Brakel, Rife & Salisbury (4); Wheat & Riggs (15); Jafar, Chapman & Casida (12)). Its magnitude is just not great enough to be clearly revealed here. First calves differed from second calves neither in average length of gestation nor in birth weight. This is attributed to the high plane of nutrition on which the heifers were kept throughout. The difference in gestation periods for seventy-three male and eighty female calves (282·74 *v.* 281·26 days) was significant ($P = 5\%$) as was also the difference in birth weights ($P < 0{\cdot}001$). Live full-term males from first and second pregnancies (69) weighed 81·7 lb. and females (73) 75·5 lb. Linking these facts were the significant regressions of birth weights on gestation periods of $0{\cdot}981 \pm 0{\cdot}261$ lb. for males and $0{\cdot}665 \pm 0{\cdot}246$ lb. for females.

Twin births

For their first and second pregnancies, the cattle in this experiment have been served for the most part by one-egg twin bulls. Two pairs were crossbreds of Ayrshire type and character. The calves they produced, supplemented by a few sired by an Aberdeen Angus bull and by a few more produced by one-egg twin heifers served by a Friesian-type pair of one-egg bulls and not in the experiment discussed above, are set out in Table 7. The figures come from three different groups of animals in chronological order. Only the first has been considered in detail in this paper.

Table 7. *Calves produced by twins and half-sibs at the Animal Breeding Research Organization and the Rowett Institute*

	One-egg twin bulls			Single-born bull		
	Cows			Cows		
Births	MZ	DZ	HZ	MZ	DZ	HZ
A.B.R.O., 1951–2						
Conceptions	58	53	46	4	3	8
Singles	54	48	42	3	3	6
Twin pairs	0	0	3	0	0	0
Abortions	4	5	1	1	0	2
Rowett, 1952						
Singles	46	12	—	0	0	—
Twin pairs	1	0	—	0	0	—
Triplets	1	0	—	0	0	—
A.B.R.O., 1952–3						
Singles	17	—	—	2	—	—
Twin pairs	1	—	—	0	—	—

Through the courtesy of Mr John Crichton of the Rowett Research Institute, Table 7 includes data from his twins served by two one-egg twin bulls that were mates of those mentioned above. Altogether five pairs of twins and one set of triplets have been produced (all two-egg or three-egg) from 252 pregnancies. This is a twinning rate of the same order as that found in large populations of dairy cattle (1–2%). It will be observed that DZ cows, which might be expected to produce twins, have in fact produced none. Three single-born HZ cows delivered one pair each but one had an injection of luteinizing hormone before final service. Of the three MZ cows to produce twins, two were at

Aberdeen, and both had been treated with luteinizing hormone just prior to conception. If these are excluded it follows that 122 matings of MZ bulls with MZ cows have produced 1 pair of twins (DZ). No support can be found therefore for the suggestion which has been made that one-egg twinning might be a fairly simply inherited character of a recessive nature. On the contrary, the data are consistent with the view that one-egg twinning is a sporadic event evoked by uterine conditions from fertilized eggs not genetically predisposed to it.

DISCUSSION

Under the conditions of this uniformity trial, the answer given by twins to the question posed at the beginning of the paper is somewhat oracular. For age at first heat and length of first pregnancies with the within-pair variance of MZ twins was about half that within DZ or HZ pairs—a result which implies that heredity contributed about half the observed variation in these characters. This would be impressive were it not for the failure of the variance ratio MZ:HZ to reach significance. Furthermore, the differences found in gestation periods did not persist when two pregnancies, instead of only the first, were studied. Heritability could have been as high as 0·5; but the data carry no assurance that it was not, in fact, zero. In length of oestrous cycle and number of services per conception there was no sign of hereditary variation. The efficiency of the twin method of experimentation depends on developing high heritabilities by reducing environmental variation within pairs. Where this cannot be achieved, the small numbers of animals under test cannot give trustworthy results. In spite of the close control of many environmental factors by virtue of the design of this experiment, no firm conclusion can be reached about the role of heredity in the expression of the characters studied, a result which tends to confirm the belief, from previous work with other methods, that only a small proportion (less than 5%) of the normal quantitative variation in the most obvious aspects of fertility is occasioned by heredity.

SUMMARY

1. Results from a uniformity trial with fifteen pairs each of one-egg and two-egg twins and pairs of half-sibs are described.

2. Intra-pair variance in age at the first observed heat was less for the one-egg than for the other pairs but not significantly so. The slowest growing pairs showed delay in onset of heat.

3. 71·8% of the intervals between heats shown by unserved heifers were 17–27 days in length. No genetic variation and no individuality could be detected in either this character or the number of services per conception.

4. The variance in the gestation period was less within the one-egg than within the other pairs but not significantly so.

5. 122 pregnancies of one-egg cows mated to one-egg bulls produced one set of two-egg twins. Two other pregnancies following therapy with luteinizing hormone resulted in twins. No twins were obtained from 60 pregnancies of two-egg twin cows mated to one-egg twin bulls.

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528. THE INHIBITION OF MICRO-ORGANISMS BY RAW MILK

II. THE SEPARATION AND ELECTROPHORETIC EXAMINATION OF TWO DIFFERENT INHIBITORY FRACTIONS

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(With 3 Figures)

INTRODUCTION

In a previous paper(1) it has been shown that two substances were responsible for the inhibition of *Streptococcus pyogenes* by raw milk. They were found to have similar properties to 'lactenin', described by Jones & Simms(2), and were called lactenin 1 (L_1) and lactenin 2 (L_2). L_1, occurring at relatively high concentrations in colostrum, is most stable at pH 6–6·5 and at this pH is destroyed by heating above 68° C. for 20 min. L_2, which is most easily observed in mid-lactation milk, is most stable at pH 7·0 and at this pH is destroyed only by heating at 74° C. for 20 min.

Jones & Simms showed that lactenin could be precipitated with the proteins of milk. They failed, however, to isolate lactenin by fractional precipitation with alcohol or ammonium sulphate, but by tryptic digestion of rennet whey, followed by dialysis with simultaneous concentration, they obtained a fraction with an activity 200 times higher than that of the original milk.

L_1 and L_2 act synergistically, each being of low potency in the absence of the other. This may account for the failure of Jones & Simms to isolate lactenin with protein-precipitating reagents.

A preliminary note on the chemical separation of the lactenins has already appeared(3). In this present paper further details and examples of the separation are given, together with the results of the electrophoretic examination of the fractions.

METHODS

Preparation of whey and concentration

Whey prepared with the aid of crystalline rennin was preferably concentrated by slow freezing and the removal of ice crystals until between one-third and one-fifth of the original volume remained, although in the earlier experiments whey was used without previous concentration.

Acetone fractionation

The method used was suggested by the work of Askonas(4).

A preliminary precipitation was carried out by adding to the whey or concentrated whey at 0° C. an equal volume of acetone in small increments, the temperature being progressively diminished to −7° C. as soon as the acetone concentration was high enough

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to prevent ice formation. The precipitate was separated in a cooled centrifuge, and then suspended in ice water, and dialysed with stirring against distilled water at 0–2° C. for 48 hr., during which time the precipitate dissolved.

For the fractionation itself, sodium acetate was added to the dialysed solution to give a concentration of 0·015 M and the pH adjusted to approximately 6·8. Acetone was added, as previously, by small amounts (see Fig. 1 for quantities) to the refrigerated liquid, but after each addition the precipitate was immediately separated by centrifugation at low temperature and taken up in 0·05 M-phosphate buffer at pH 7. The fractions obtained were then assayed for their content of L_1 and L_2.

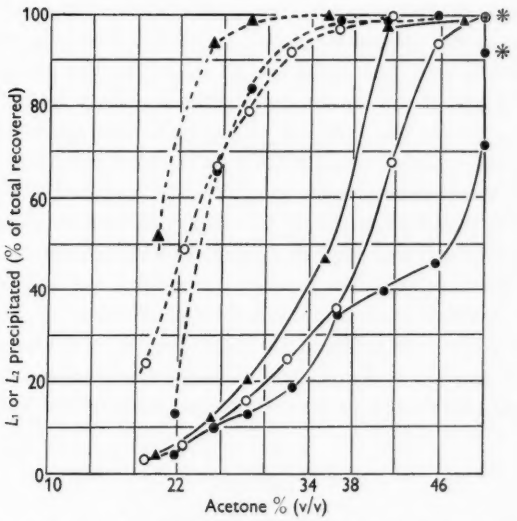

Fig. 1. The proportion of L_1 and L_2 in the precipitate produced at various concentrations of acetone. ———, L_1; – – – –, L_2; ▲, Exp. 1; ○, Exp. 2; ●, Exp. 3. * $CaCl_2$ added to a final concentration of 0·001 M

Assay of L_1 and L_2

The fractions generally contained enough acetone to ensure their sterility, though not enough to interfere with the assay. The samples without acetone were heated at 60° C. for 20 min. before the assay.

The method of assay of L_1 and L_2 has already been described (1). For the assay of L_2, a colostrum containing 1500 units of L_1 per ml. was added to the diluent to give a concentration of 0·25%, and for the assay of L_1, a milk heated at 70° C. and containing 50 units of L_2 was added to the diluent to give a concentration of 5%. The standard was the same as in the experiments already reported (1). The assays were generally done in duplicate.

Electrophoresis

This was carried out in the Tiselius apparatus with capillaries fitted to the U-tubes, by the technique used for preparative electrophoresis (see Svensson (5)). The whole system was completely closed except for compensator openings at the top of each U-tube arm, fitted with 3-way taps. A capillary intake to the bottom of the U-tube and two capillary exits from each arm were kept closed during the run. One of the compensator

openings was left free to the air to allow for small volume changes which might occur during the run. The lactenin fractions which had been dissolved in 0·05 M-potassium phosphate buffer at pH 7·0 were dialysed against repeated changes of it at 0° C. for approximately a week, before electrophoresis in this same buffer. After electrophoresis, the liquid in each arm of the U-tube was expelled through the adjacent capillary without mixing and broken up into a number of serial samples, each corresponding to a layer 3–5 mm. thick from the U-tube. Electrolytically generated gas was used to force fresh liquid into the system to displace the required samples through the exit capillary at a rate of 0·04–0·05 ml./min. The liquid was brought from the exit capillary to the sampling point through fine polythene tubing as suggested by the technique of Crook & El-Marsafy (6). Preliminary experiments with potassium permanganate and then with starch solutions were carried out to test the present technique, and it was found, for example, that where the boundary in the U-tube was about 4 mm. thick, the last sample from above the boundary contained 5% of the lower solution, the boundary itself 53% and the next sample 90%. The technique was therefore judged to be adequate for the purpose of this work. It is theoretically possible to calculate from the total volume of expressed fluid the original position in the U-tube of each sample. It is, however, difficult to make such a complex piece of apparatus completely free from leaks and it was found desirable to check the calculated position of samples against photographs of the diagram taken at definite stages of the sampling. Discrepancies of several millimetres were found. The photographs were therefore used to correct the calculated positions of samples before expulsion. This correction was applied only to the results calculated for L_1 (Fig. 3), but although it was not applied to Fig. 2, and although the abscissae may therefore be in error by several mm., the boundary separations are so great that useful conclusions can still be drawn.

RESULTS

Acetone fractionation

The results of several acetone fractionations are summarized in Fig. 1, from which it is clear that, in spite of variations, the precipitation of L_2 is always almost complete before that of L_1 begins. The variations between experiments probably arose because the control of such variables as ionic strength and temperature, to both of which the precipitation is very sensitive, was not sufficiently rigid. Furthermore, the acetone concentration was calculated from the original volume of solution, that (variable volume) removed by the precipitates being neglected. Since, however, the desired end, namely the separation of L_1 and L_2, was being achieved, no attempt was made to prevent these relatively minor variations.

The ordinates in Fig. 1 show the proportion of the total *recovered* activity precipitated at each stage of the fractionation. To complete the picture these totals of recovered activity are recorded in Table 1 as percentages of the activity in the dialysed solution of the precipitate obtained in the first stage when an equal volume of acetone had been added to whey. They give therefore the yield of the fractionation process.

It appears from Fig. 1 that fractionation between say 22, 35, and 50% acetone should give a useful separation of L_1 and L_2. This was tried a number of times without success. Table 2 shows examples of such results, which seem to indicate that it is essential to carry out the fractionation by small steps.

Table 1. *Percentage yields of the fractionation process (see text)*

	L_1	L_2
Exp. 1	92 (39,000 units)	96 (40,000 units)
Exp. 2	93 (111,000 units)	87 (69,000 units)
Exp. 3	95 (103,000 units)	140 (154,000 units)

For definitions of units see Auclair & Hirsch (1).

Table 2. *The short fractionation of the lactenins*

% acetone (v/v)	Activity precipitated (% of total recovered) Exp. 1 L_1	Exp. 1 L_2	Exp. 2 L_1	Exp. 2 L_2
22	0	3	11	72
35	88	99	82	100
50	100	100	100	100

Electrophoresis

Although the separations shown in Table 2 were not good, the first sample of Exp. 2 contained 72% of the L_2 against only 11% of the L_1 and this fraction was used for electrophoresis. The results are given in Fig. 2, which shows a tracing of the electrophoretic diagram at the end of the run, i.e. after 345 min. at 12 mA., and a plot on the

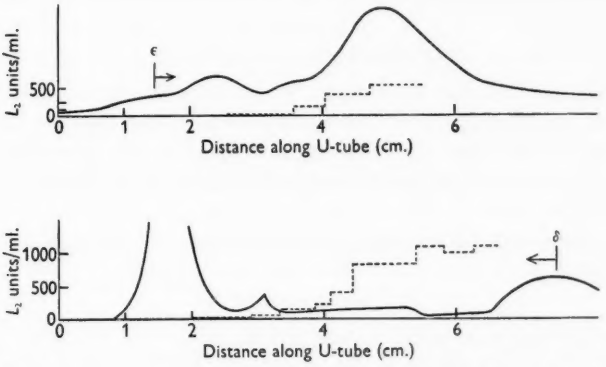

Fig. 2. Electrophoretic diagram, ————, and biological activity, – – – –, for L_2.

same horizontal scale of the biological activity of expressed samples against their calculated position in the U-tube. Fig. 2 shows that, on the descending side, L_2 is not separated from the main peaks, but on the ascending side, it is distinctly separated from the main protein components and is probably identical with a minor component that spreads very markedly. These results suggest that it should be possible to obtain far more potent preparations of L_2.

For the electrophoresis of L_1, the last three precipitates of the second experiment of Fig. 1, being free from L_2 which had already been precipitated, were bulked and prepared as described above. The electrophoresis was carried out as for L_2, the run being terminated after 310 min. at 12 mA. Sampling was carried out in the same way, but the calculated positions were corrected from a series of photographs taken during the sampling. The

results are shown in Fig. 3, from which it is clear that L_1 probably corresponds to a well-marked peak of relatively small area. Again separation on the descending side is poor, and again this substance also should be capable of considerable further purification. A highly anomalous effect must be noted. Lactenin 1 is the slowest boundary on the ascending side, but the fastest on the descending side, an effect worth further investigation. The 'B' value(5) of the dilute phosphate buffer is far too low for quantitative electrophoresis, but it was chosen because the resolution of peaks is often better in such buffers(7).

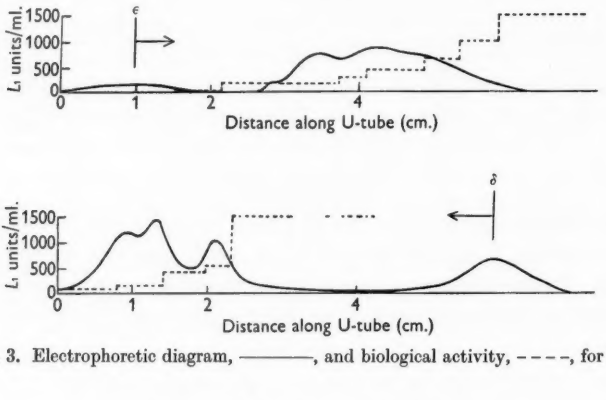

Fig. 3. Electrophoretic diagram, ————, and biological activity, – – – –, for L_1.

SUMMARY AND CONCLUSIONS

The two substances previously assumed to be necessary for the bacteriostatic action of raw milk have been concentrated and separated from one another. Electrophoretic experiments have shown that the preparations, though highly active, are still far from pure and that it should be possible to obtain material of much greater potency.

The authors wish to thank Miss Grace Thorner for technical assistance.

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529. PULSATION AND MILKING RATE

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The pulsator mechanism of the double-action milking machine causes the rhythmical expansion and collapse of the rubber teat-cup liner during milking by alternately applying a partial vacuum (usually 13–15 in. Hg) and admitting atmospheric air to the space between the rubber liner and the teat-cup shell. The inside of the liner is subjected to continuous partial vacuum.

With similar conditions of partial vacuum inside and outside its wall the liner assumes its normal tubular shape, the teat orifice is opened, the milk is drawn out and flows down the milk tube into the milk bucket.

It is generally believed that when the chamber surrounding the liner is under atmospheric pressure, the liner collapses round the teat and closes the orifice, whilst below the teat the liner walls meet cutting off the vacuum of the milk tube from the teat. This movement of the rubber liner, which prevents milk flow, has a massaging effect on the teat and aids in relieving the congestion of blood caused by the force of the partial vacuum with the liner in the expanded position.

Under practical conditions milk flow may not entirely cease during the collapse phase of the pulsation cycle. This will depend on the size and shape of the teat, the tonus of the sphincter muscle, the design and adjustment of the liner and the physical properties of the rubber of the liner.

Machine milking without pulsation, by retaining the liner in the expanded position, should result in higher rates of milking. However, this is not a practical method of milking, except when milk is flowing freely from the teat (1), because the painful sensation of the constant partial vacuum on the teat may inhibit milk ejection and damage the tissues of the teat.

Some experimental work has been carried out to determine the effect of changes in the expansion: collapse ratio (pulsation ratio) of the pulsation cycle and changes in the number of cycles per minute (pulsation rate) on machine milking rate, but the findings of different workers are contradictory. Smith & Petersen (2) found that raising the pulsation ratio from the normal 50:50 to 60:40 and 75:25 gave a slightly increased rate of milking. On the other hand, Whittleston & Verrall (3) reported that neither changes in the pulsation ratio nor pulsation rates of 21, 42 and 84 cyc./min. affected milking rate.

In the absence of evidence indicating that milking rate is affected by changes in pulsation rate, it is generally agreed that rates of from 40 to 50 cyc./min. are most suitable. At such speeds pulsators operate evenly, stimulation of milk ejection is satisfactory and little trouble is experienced with teat-cups falling from the teats.

The object of the experiments reported below was to determine the effect on milking rate of varying the frequency of pulsation using more accurate experimental methods than those previously used. This study is the first in a series designed to investigate the importance of pulsation in machine milking and the form in which it should be applied.

Exp. I. The effect of pulsation rates of 20, 50 *and* 80 *cyc./min. on machine milking rate*

Plan of experiment

The nine cows used were divided into three groups with one relatively slow, one medium and one relatively fast milker per group.

Each group of cows received each of the three experimental treatments of 20, 50 and 80 pulsation cyc./min. (see Table 1).

Table 1. *Plan of experiment showing the experimental treatment (i.e. pulsation rate) which the cows received during the three experimental periods*

		Experimental periods		
Group	Cows	1	2	3
I	1, 2, 3	50	20	80
II	4, 5, 6	80	50	20
III	7, 8, 9	20	80	50

An initial conditioning period of 4 days when all nine cows were milked at 50 pulsations/min. was followed by the three 7-day experimental periods. At the morning milkings of the 4th, 5th, 6th and 7th days of each experimental period speed of milking records were obtained for each cow in the manner described by Foot (4). The mean value of measures of milking rate obtained from these four records for each cow in each group were used in comparing the effects of the three different pulsation rates on the rate of milking.

Throughout the experiment a single Alfa-Laval bucket unit, with Alfa-Laval hooded-mouthpiece teat-cup liners (type no. 24803 B) was operated at a vacuum of 15·2 in. Hg.

Three Alfa-Laval pulsators (type no. 20750), giving pulsation ratios of 50:50, were used in the experimental periods and were set to give 20, 50 and 80 pulsation cyc./min. respectively. The vacuum level and pulsation rates were checked at each milking and adjusted when necessary.

Results

From the milk-flow records obtained at the four timed milkings in each experimental period six measures of milking rate were extracted. The mean values for these measures calculated for each cow and for all nine cows are listed in Table 2, and indicate that the progressive increases in pulsation rate gave faster milking. The mean effects of the changes in pulsation rate from 20 to 80 cyc./min. were increases of 1·92, 1·39 and 0·34 lb./min. in the rate in the first minute, peak flow and overall rate respectively, and a decrease in the total duration of milking of 1·26 min.; all these effects being significant at the 5% level of significance.

Reference to the individual figures for each cow indicates that whereas all nine cows showed the increase in peak flow and rate in the first minute, the effect on total milking time was not apparent for three of the cows.

Table 2. *The effect of milking at three different pulsation rates on measures of machine milking rate*

Measure of milking rate*	Pulsation rate/min.	Cow 1	2	3	4	5	6	7	8	9	Mean of nine cows
(*a*) Morning milk yield (lb.)	20	16·35	16·53	26·78	20·28	17·90	20·05	24·20	24·43	20·63	20·79
	50	18·03	16·98	26·58	20·83	18·23	19·23	23·73	16·50	20·20	20·03
	80	15·08	14·15	26·05	18·88	19·48	20·10	23·62	18·83	21·60	19·73
(*b*) Total time (min.)	20	10·54	6·63	8·18	6·78	5·63	6·50	7·65	8·76	8·81	7·72
	50	9·80	6·05	7·09	6·85	5·93	6·05	7·11	6·55	6·80	6·91
	80	7·94	6·68	6·33	5·84	5·93	6·58	6·14	6·27	6·45	6·46
(*c*) Stripping yield (lb.)	20	2·08	1·50	0·53	0·80	0·78	2·50	0·55	0·48	0·88	1·12
	50	2·38	1·88	0·85	0·98	0·75	2·00	1·10	0·78	0·45	1·24
	80	2·00	1·35	0·75	1·25	0·75	1·75	1·30	1·03	0·60	1·20
(*d*) Rate in 1st min. (lb./min.)	20	2·05	4·60	4·08	4·95	4·53	5·38	4·93	3·48	3·30	4·14
	50	2·43	5·98	5·40	6·23	6·18	6·75	6·80	3·75	5·60	5·46
	80	2·58	6·40	5·78	6·40	7·20	7·28	7·53	5·50	5·83	6·06
(*e*) Peak flow (lb./min.)	20	2·70	4·68	5·25	6·28	6·33	7·22	6·03	4·60	5·23	5·37
	50	3·35	6·00	6·80	6·85	6·88	7·33	7·10	3·98	7·18	6·16
	80	3·45	6·40	7·53	7·10	7·53	7·98	7·70	5·57	7·58	6·76
(*f*) Overall rate (lb./min.)	20	1·55	2·49	3·28	2·99	3·18	3·08	3·16	2·79	2·34	2·76
	50	1·84	2·81	3·75	3·04	3·08	3·18	3·34	2·52	2·97	2·95
	80	1·91	2·12	4·12	3·20	3·29	3·06	3·85	3·01	3·35	3·10

* For definitions of terms see previous paper (5).

Changes in the pulsation rate did not appear to affect total milk yield and strippings yield, and the differences in these factors which occur are small and not statistically significant.

Exp. II. To determine whether the increases in milking rate found in Exp. I were due to the reaction of the cows to the stimulus of the higher pulsation rates or to changes in the mechanical conditions in the teat-cups

For this investigation a milking machine was constructed which milked the left side of each udder separately from the right side. This machine was used to apply pulsation at different rates simultaneously to the two sides of the udder. In this way it was possible to eliminate the effect of milk ejection which is the only known way in which the reaction of the cow influences milking rate.

Description of the experimental milking machine

The machine was essentially a series of eight two-way cocks made by drilling at intervals along the length of the cylindrical body and plug of a large control cock. Rotation of the lever fixed to one end of the plug through an arc of 90° switched all of the two-way cocks simultaneously.

At the claw-piece the milk and pulsation lines for the two sides of the udder were provided with separate channels. The two milk lines from the claw were connected to two connexions on the inlet side of the control cock, and by switching the lever the milk was diverted through the control cock to either one of two pairs of jars. The partial vacuum system was connected to the jars through the control cock so that when the machine was in use only one pair of jars was connected to the main vacuum system. The other jars could then be disconnected, weighed, emptied and replaced.

To facilitate the removal of the lids of the jars, and to ensure that every jar was vacuum

tight before being reconnected to the milk line, a small two-way cock was fitted to each lid. One side of the cock, used when removing a full jar, opened to the atmosphere, and the other, used when fitting an empty jar, connected with the vacuum system by a fine-bore tube.

The pulsation tube from each side of the udder was connected via an auxiliary two-way cock to the inlet side of the control cock and the outlet sides to two Alfa-Laval pulsators (type no. 20750).

By changing the position of the control cock and auxiliary cocks during milking it was possible to milk both sides of the udder at the control pulsation rate, both sides at the experimental rate or one side at the control and the other at the experimental rate. In addition, the yield of milk obtained from each half-udder when milked under the different pulsation conditions could be measured.

The experimental milking machine was fitted with Alfa-Laval stretched liners (type no. 20003B).

Plan of experiment

The five cows selected for this experiment had a wide range in milking rates: their peak flow rates in early lactation varying from 1·95 to 7·03 lb./min. Throughout the experiment they were milked at 12 hr. intervals at a partial vacuum of 15·3 in. Hg.

The experimental milkings were preceded by a 7-day conditioning period when all milking was carried out at the control pulsation rate of 50 cyc./min. The experimental period lasted 8 days, for the first 4 days the experimental pulsation rate was 80 cyc./min. and for the next 4 days 20 cyc./min.

During the 8-day experimental period each milking commenced with both sides of each udder subjected to 50 pulsations per minute. This was continued until the milk appeared to be flowing into the jars at a constant rate, a period which varied from 15 to 45 sec. Following this the period of constant flow was divided into four equal intervals, the duration of which had been determined in initial trial milkings. These intervals varied from 20 to 60 sec. because of the differences in the yields and milking rates of the five cows.

Table 3. *The pulsation rates applied to each side of the udders of the five cows in the four recorded intervals of each milking*

	Morning milking		Evening milking	
Interval	Left quarters	Right quarters	Left quarters	Right quarters
1	50	50	50	50
2	E	50	50	E
3	50	E	E	50
4	50	50	50	50

The pulsation rates applied for each cow at each of the experimental milkings are given in Table 3.

The experimental pulsation rate, *E*, was 80 cyc./min. for the first eight milkings and 20 cyc./min. for the next eight milkings.

The yield of milk, measured in grams, obtained from each side of the udder in each of the four intervals was recorded separately.

Results

In Table 4 the total yield of milk obtained from the half-udders when milked at the experimental pulsation rate in the milking intervals 2 and 3 is expressed as a percentage of the yield at the control rates in these intervals.

Table 4. *The rate of milking at* 80 *and* 20 *pulsations/min. expressed as a percentage of rate of* 50 *pulsations/min.*

Cow no.	Milkings 1	2	3	4	5	6	7	8	Mean
				(*a*) At 80 pulsations/min.					
1	105·7	116·6	118·8	114·9	105·0	120·9	111·5	117·2	113·85
2	113·6	106·4	104·7	110·1	120·2	110·6	113·5	109·5	111·08
3	107·0	108·1	107·7	103·9	108·2	104·9	106·0	107·6	106·68
4	110·1	106·1	116·4	106·8	118·1	110·2	117·0	107·0	111·46
5	113·4	101·8	106·9	95·4	112·6	112·0	102·3	99·7	105·51
								Mean	109·72%
				(*b*) At 20 pulsations/min.					
1	82·1	84·6	79·8	84·5	81·3	84·2	80·9	86·9	83·04
2	76·0	74·2	79·1	68·7	73·4	76·6	78·3	83·2	76·19
3	83·0	83·8	75·7	75·6	80·2	75·0	82·4	82·2	79·74
4	85·0	73·4	76·9	75·5	84·9	75·5	85·0	84·0	80·03
5	81·7	73·1	82·1	80·9	93·8	86·2	92·1	102·0	86·49
								Mean	81·10%

For thirty-eight out of forty milkings 80 pulsations/min. gave faster milking than 50 pulsations/min., and for thirty-nine out of forty milkings 20 cyc. gave slower milking. Using 50 cyc./min. as the standard, the mean relative milking rates at 20 and 80 cyc./min. were 81·1 and 109·7% respectively.

The three abnormal readings were all obtained from the same cow, and it is believed that they were due to a trend in the milking rate of one side of the udder during milking while the rate of the other half remained constant.

DISCUSSION

The results of these two experiments indicate that under the conditions described milking rate increases with increase in pulsation rate from 20 to 80 cyc./min. The fact that the relative peak flow milking rates at 20 and 80 cyc./min., compared with 50 cyc./min., were 87·2 and 109·7% in Exp. I and 81·1 and 109·7% in Exp. II suggests that the increase was mainly due to changed mechanical conditions in the teat-cup, since the effect of milk ejection was eliminated by the design of the second experiment.

Because the mechanical action of pulsation appears to have an important influence on milking rate it will be necessary in future experimental work on pulsation to present records of the pulsation characteristic (i.e. a graphical record of the changes in the partial vacuum throughout the pulsation cycle) at the pulsator and in the pulsation chamber of the teat-cup during milking. At the same time any changes in vacuum conditions at the teat orifice must be measured. If a pulsator is incapable of evacuating the pulsation system during each cycle changes in pulsation rate will not have the effect measured in these experiments.

The results given in this paper are of practical significance in that they were obtained with standard milking machine parts and are likely to apply under most farm conditions

with standard makes of machine. It would appear that milking times can be reduced by about 10% per cow by milking at a rate of 80 pulsations per minute instead of the usually recommended rates of 40–50 pulsation cycles per minute.

We wish to thank Mr A. S. Foot for his guidance and encouragement and Mr H. S. Hall for advice and engineering facilities in preparing the experimental equipment.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

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It is now some 6 years since the appearance in this series of a Review of Bacteriology and Mycology applied to Dairying (1) with sections on milk control, detergents and disinfectants, and pasteurization. Nearly 12 years have elapsed since a complete Review (2) including all the sections listed above was published in the *Journal of Dairy Research*; some attempt has been made to deal with the more important work going back to these Reviews.

The death of Dr Robert Burri last year has meant grievous loss to bacteriology the world over.

I. GENERAL

The new books of interest to dairy bacteriologists, apart from those mentioned under subsequent headings, include a tenth edition of Zinsser's *Textbook of Bacteriology* (3), a third edition of Dible's *Recent Advances in Bacteriology* (4) by MacLennan & Barber, a ninth edition of the American *Standard Methods for the Examination of Dairy Products* (5), and Werkman & Wilson on *Bacterial Physiology* (6). Two books from America, *Industrial Microbiology* (7) and the second edition of *Microbiology, General and Applied* (8) have points in common, including sections dealing with milk and milk products. Bisset & Moore's *Bacteria* (9) approaches micro-organisms from the standpoint of natural history, morphology and evolution; two books by Davis have appeared recently—*A Dictionary of Dairying* (10) and *Milk Testing* (11). *The Yeasts: a Taxonomic Study* (12) by Lodder & Kreger-van-Rij deserves particular mention, and bids fair to become a classic in the manner of Bergey's *Manual* (13). The new annual journal, *International Review of Cytology* (14) also deserves mention, with the bimonthly *Applied Microbiology* (15) published under the sponsorship of the Society of American Bacteriologists and embracing the application of microbiology to all fields of industry, food, sanitation and agriculture other than animal and plant disease. Capstick (16) has reviewed developments in the dairy industry during the last century.

New apparatus or techniques and new uses or ways of using familiar apparatus are referred to later, where appropriate, but some may be mentioned here. Bonifas & Novel (17) have used a special type of haemocytometer, together with a phase-contrast microscope for direct counts of bacteria, thus, they claim, obtaining more accurate results partly by obviating the difficulty of assessing the number of individual bacteria in chains or clumps. Williams (18) has devised a means of counting bacteria in suspensions using cast agar films. 'Biochemical methods for bacteriology' have been described by Clarke & Cowan (19) in which many of the vagaries of cultural techniques may be avoided by the use of thick suspensions of bacteria; these, with their

preformed enzymes, are added to chemically defined substrates, giving clear-cut results. A clockwork automatic bacteriological inoculating device has been devised by Fuson & Marcus(20). An ingenious device(21), patented in the U.S.A., for counting bacterial colonies, consists of a fountain pen connected to an electro-magnetic counter which registers each time the pen touches the Petri dish or roll-tube. Hannay(22) has described a new type of laterally illuminated colony counting chamber. British Standards for Petri dishes(23) have been issued recently. A microtechnique for the growth of many cultures, particularly for assay work, in one Petri dish has been developed by Turner-Graff(24). Newbould's(25) roll-tube spinner is designed to use cheap, easily obtainable vials or bottles. A suggestion contributing, in the long run, to economy is that of using rigid, seamless tubes of Kraft paper instead of the paper wrappings commonly used for individual sterile pipettes(26); Lack(27) has described a hot box for micro-cultures, which dispenses with a water jacket since the heating element consists of glass electro-thermal heating tape. From America have come details of a simple large-scale anaerobic culture apparatus(28) utilizing a milk can as the main container, and of the uses to which spun glass(29) may be put as an air-filtering medium for bacteriological purposes.

The text of the papers read, and the discussion, at a Symposium of the Institute of Biology in London in 1951 have been published under the title *Freezing and Drying*(30); diversity of opinion and lack of collaboration and understanding between bacteriologist and vacuum engineer was apparent concerning even such vital factors as the plugs for drying tubes. Fry & Greaves(31), working mainly with a paracolon bacillus over a 3-year period, have shown that the nature of the suspending medium is one of the most important factors determining the survival rate of bacteria dried *in vacuo*; the addition of 5–10% of glucose or lactose increased the percentage survival. Young cultures were less resistant to drying than older cultures, but the cell concentration appeared to be of little consequence. 'Mist desiccans' (1 part broth, 3 parts serum with 7·5% added glucose) proved a satisfactory suspending medium for sensitive organisms such as *Neisseria gonorrhoea*. Briggs' observations(32) on the freeze-drying of lactobacilli are in almost complete agreement with those of Fry & Greaves; using strains of *Lactobacillus acidophilus* and *L. bulgaricus*, which were particularly sensitive to freeze-drying, she obtained with her suspending media survival rates of over 10% after 18 months' storage; her work is being continued with other species of lactobacilli subsequently tested to determine whether selection has taken place, although this should not be a danger except where very low survival rates are experienced. Sharpe(33) has tested the precipitin and agglutination reactions of cultures of *L. acidophilus* and *L. bulgaricus* which had been freeze-dried and stored for 3 months, finding no differences from those of the original cultures; the vitamin B_{12} requirement of a strain of *L. leichmannii* was unaltered after freeze-drying and storage for 1 month(34); similar observations with *L. arabinosus* relate to a storage period of 3 years(35). Further observations on the retention of original characteristics by bacteria after freeze-drying and storage have been made by Fasquelle & Barbier(36) (staphylococci and pneumococci), Ungar(37) (mycobacteria), Nymon, Gunsalus & Gortner(38) and Gortner & Volz(39) (*L. arabinosus* and *L. casei*). Proom & Hemmons(40) and Rhodes & Fisher(41), in work on large numbers of bacteria representing several genera, have shown that survival rate varies to some extent with species. Titov(42) has described his methods of freeze-drying lactic acid bacteria, and Guygo(43) has outlined the principles concerned and given formulae for the calculation of the different factors involved. Directories of British Commonwealth Collections(44, 45) of micro-organisms have been published recently. A new departure in the field of freeze-drying concerns its application to electron microscopy; Williams(46) has described a method of freeze-drying for electron microscopy which he and Fraser(47) have used in their studies of the serologically related T_3 and T_7 bacteriophages. These are unique among the *T* phages in appearing as tail-less spheres; however, by electron microscopy after freeze-drying they were shown to possess short, stubby tails, which were not considered to be artefacts. Methods and principles of freeze-drying were dealt with by Proom(48) at a recent Symposium of the Society for Applied Bacteriology entitled 'The effect of cold on micro-organisms'; other contributions were made by Mattick(49) (introduction), Davis(50) (dairying)

and Ingram(51) (food). In the new journal, *Applied Microbiology*, Hartsell(52) records his experiences in the preservation of bacterial cultures under paraffin oil, which he claims is a practicable method.

Closely related to temperature and drying is the phenomenon of sporulation in bacteria. The subject has been covered exhaustively in a Symposium(53) on the biology of bacterial spores held at the annual meeting of the Society of American Bacteriologists in 1951. Schmidt(54) has described a method of determining the thermal resistance of bacterial spores, and Williams & Zimmerman(55) have determined the heat resistance of both vegetative cells and spores of *Bacillus cereus*, *B. subtilis*, *B. mycoides* and *B. mesentericus*. The activating effect of sublethal temperatures on spore germination reported in 1945(56) has been confirmed by von Gavel (57) with a strain of *B. subtilis*, isolated from milk. Saturated fatty acids have been found to be anti-sporulation factors in organic media(58).

Messerschmidt(59) has recognized the variations obtained with different brands of peptone(60), and recommends that an indication of the protein source and method of preparation should be given. Bogdanov(61) has described in detail the preparation of media for total counts, proteolytic bacteria, lactic acid bacteria, lipolytic organisms, yeasts and moulds. A plating method, incorporating sodium thiogallate, has been described for propionic acid bacteria(62). Counts of bacteria in the presence of large numbers of yeasts may be made on a synthetic agar medium containing a fungistatic antibiotic(63). It is suggested by Pelczar & Brown(64) that a synthetic medium known as 'S_4', made up of a number of different amino-acids, vitamins, purines and pyrimidines, might be used as a reference standard against which different peptone media could be evaluated. A method for the detection of butyric acid bacteria in the vegetative form has been described by Lind(65).

The position of *Streptococcus bovis*, once confused with the lactic streptococci, in serological group D has been confirmed by Shattock(66), and Sharpe & Shattock(67) have divided group D strains from neonatal diarrhoea into two broad groups—*Str. faecalis* and *Str. bovis* and *durans*. Sharpe(68, 69) has continued her work on group D streptococci from infants' faeces, and has shown a high correlation between their biochemical characteristics and tyrosine decarboxylase activity. A streptococcus producing a yellow pigment, isolated from the teat of a cow by Hannay(70), proved to be a group D strain closely related to *Str. faecalis* but differing from it in fermenting raffinose. Certain streptococci of groups D and N have been shown by Sharpe(71) to share a common antigen; this is of interest in connexion with Briggs & Newland's(72) observations on the serological typing of the lactic streptococci (see p. 384). Berridge & Briggs(73) have made electrophoretic examinations of chemically isolated, serologically specific material from organisms of different streptococcal groups, including N; although interesting differences were observed in the electrophoretic diagrams of the various groups, the method was found to be uneconomic and less satisfactory than others (serological) already available. A method(74) which has been used successfully for the separation of *Str. lactis* from *Bacterium coli* involves the inactivation of bacterial cells by heat or mechanical pressure, and their subsequent reactivation by treatment with phenol or glycocoll. Infra-red spectrophotometry has received attention in recent years as a means of identifying and classifying biological materials(75, 76, 77, 78), since the infra-red absorption spectrum of a specimen is a function of its chemical composition. The technique has now been applied to extracts of *Mycobacterium tuberculosis*(79), and to a variety of whole organisms of different genera by Stevenson & Bolduan(80) with considerable success and promise for the identification of both species and strains. The incidence, properties and significance of Actinomycetes in milk have been studied extensively by Hlaváčková(81), and the biochemical activity of coliform organisms in milk by Gopalakrishna & Iya(82).

A review by Möller of the fields of nutrition and microbiological assay in relation to lactic acid bacteria is mentioned on p. 384, and the same author(83) has also written recently a more general review of the subject. Three papers dealing in this connexion with lactic acid bacteria may be mentioned: Toennies & Frank(84) have studied the role of pH and buffering capacity of the medium in the growth of *Str. lactis* and *L. casei*, MacLeod & Snell(85) metallic ion antagonism

in lactic acid bacteria, while a new disaccharide, 'Leucrose', has been shown to be formed in small quantities by *Leuconostoc mesenteroides* during the synthesis of dextran from sucrose(86).

II. LACTIC ACID AND ALLIED BACTERIA

A comprehensive bibliography on lactic acid and its derivatives, mainly concerning chemical and industrial aspects, has been compiled by Fisher, Dixon & Filachione(1). Tittsler, Pederson, Snell, Hendlin & Niven(2) have contributed to a Symposium on the lactic acid bacteria in which most aspects of the subject were considered. Abd-el-Malek & Gibson have continued their studies in the bacteriology of milk(3, 4), and have named a Gram-negative rod from pasteurized milk *Alkaligenes tolerans*; this is distinguished from *Alk. viscosus* by its survival at 63° C. for 30 min. in milk, and by its inability to attack natural fats. These authors have adopted the name 'Corynebacterium' for the saprophytic highly heat-resistant bacterial rods from milk and dairy equipment, in preference to the 'Microbacterium' of Orla-Jensen; these thermoduric organisms appeared to form a homogeneous group identified as *Corynebacterium lacticum*. Gorini(5) has suggested a scheme for the enzymatic grouping of organisms, particularly the lactic acid bacteria, according to their glycolytic, proteolytic or acidoproteolytic properties; Williams & Campbell(6) have described a test for the detection of heterofermentation by lactic acid bacteria. A pamphlet(7) from the Netherlands contains practical hints on the manufacture of yoghourt. The degree of inhibition of *Bact. aerogenes* by *Str. lactis* in milk has been shown to be partly a function of temperature(8); Ritter(9) has found that *Bact. aerogenes* can inhibit the growth of *Bact. coli* and indicates that factors besides acidity are concerned.

The ('lactic') streptococci of serological group N have received considerable attention in recent years, particularly from a taxonomic point of view; Briggs & Newland(10, 11) have succeeded in preparing a specific group N precipitating serum for *Str. cremoris*, and Swartling(12) has shown that strains such as *Str. citrophilus*, *Str. lactis aromaticus*, *Str. diacetyl aromaticus* and probably *Str. diacetilactis*, which utilize citrate in the production of CO_2, volatile acids and C_4-compounds (diacetyl, etc.), also belong to serological group N. His observations have been confirmed by Briggs(13) with *Str. diacetilactis*. The type antigen structure of the lactic streptococci has also been studied by Briggs & Newland(14), who found that strains of each of the named 'varieties' possessed a common specific type antigen; in view of the commercial use of the antibiotic nisin (see later) the demonstration of a type antigen specific for the 'inhibitory' strains of *Str. lactis* may be of value. In addition to their specific type antigens, the different varieties were also found to contain heterologous antigens with complicated overlapping patterns, and the 'classic' strains of *Str. lactis* OJ and *Str. cremoris* HP were shown to possess two common type antigens, in inverse proportions of 4:1/1:4. (Sharpe(15) has demonstrated the presence of a common type antigen in certain streptococci of serological groups D and N.) Hirsch's(16) observations on 'wild' strains of *Str. cremoris* from milk produced in the Jura mountains of France, coupled with Briggs's and Newland's findings, suggest that *Str. lactis* and *Str. cremoris* can no longer be considered as mere mutants of a single original variety. However, the question of species rank in the group of lactic streptococci still demands consideration, particularly as new names or varietal descriptions(17, 18, 19) continue to be added to the list. An interesting and provocative paper on the possible mode of evolution of the lactic streptococci has been written by Hirsch(20). Gibshman(21) has given detailed descriptions of medusa-head colonies of *Str. lactis* and *Str. diacetilactis* on meat peptone agar; the cells forming such colonies divided cross-ways and length-ways, and showed clustering. Of 3000 cultures isolated from milk by Nelson & Thornton(22) in Canada, 7·9% were lactic streptococci; 4% of these were *Str. cremoris*, the remainder *Str. lactis*, and the highest incidence of lactic streptococci was in spring and summer milk.

An excellent review by Möller(23) covers most of the salient literature on the nutrition and nutritional requirements of lactobacilli. A medium supporting good growth of all species has recently been developed by Briggs(24) (p. 400) who used it as a substrate for physiological and

biochemical tests for the classification of lactobacilli(25). In a collection of 452 strains eight groups were defined on the basis of production of gas from glucose and citrate and of ammonia from arginine, growth at 15, 45 and 48° C., heat survival at 60 and 65° C., and tolerance of 4, 6 and 8% NaCl; 390 (86·3%) of the 452 strains were classified. Sharpe's(26) serological work on the same collection of lactobacilli has so far shown a good measure of agreement with these results. Briggs's observations on the freeze-drying of lactobacilli(27) have been described already (p. 382). A semi-synthetic medium for lactobacilli has also been described by Rogosa, Mitchell & Wiseman(28), and in Emard & Vaughn's(29) work the selective inhibitory action of sorbic acid proved more effective for the isolation of lactic acid bacteria (and clostridia) from mixed culture sources than the addition of special nutrients to a basal medium. Variations in environmental conditions and in the composition of media have been found to affect the morphology of colonies of some lactobacillus species(30). von Plotho(31) suggests (on morphological grounds only) that *L. bifidus* should be re-classified as *C. bifidum*. Coolidge(32) reports changes from hetero- to homo-fermentation in single-cell cultures of lactobacilli.

Starters

Hales(33) has described the different types of starter cultures used in cheese-making, their preparation and maintenance, and also the effects of bacteriophage on starters. In review articles Czulak(34) and Demeter(35) have discussed the factors, including bacteriophage, which influence starter activity. The problem of bacteriophage, and of antibiotics and other inhibitory substances in milk is dealt with later. Media for mother cultures of starter organisms, in one of which a strain of streptococcus was maintained in an active condition for 2½ years, have been described by Alais & Brigando(36); Horrall, Elliker & Kensler(37) have found that the activity of starters grown in a medium consisting of spray-dried skim milk (10%) in distilled water varied less from day to day than that of the same cultures grown in whole milk. It is claimed that the rate of acid production by lactic acid bacteria is increased by the addition of 0·0001–0·001 M-sulphite (SO_2 or an alkali sulphite) to milk media(38), and Talce(39) has shown from results based on the examination of 386 starters that good quality milk is essential for the production of good quality starters. Tests for starter activity and aroma formation have been described using resazurin(40) and O'Meara's creatinine test(41); the factors affecting such tests have been discussed by Golding, McCorkle & Milich(42). Determination of volatile acidities is not alone sufficient to detect coliform contamination of starter cultures(43).

Bacteriophage

The problem of bacteriophage in the dairy industry has been reviewed by Elliker(44), Parmelee, Nelson, Turner & Carr(45), and Overby(46); the latter author has made interesting observations on a starter phage which failed to pass through the Seitz filter, and was shown by electron microscopy to be nearly twice the size of previously reported phages. By means of the electron microscope Shew & Hodge(47) have shown that the phage specific for the HP strain of *Str. cremoris* is morphologically similar to that of coliform and other bacteria. According to Wilkowske(48) morphology and plaque size are not reliable criteria for the differentiation of phages, but he was able to divide fifty-one phage preparations into seven groups by serological means, cross-phage tests and heat resistance. On morphological grounds alone, however, Williamson & Bertaud(49) claim to have observed a new phage strain active against *Str. cremoris*, which was also of large size, and of different shape from all phages so far described. The effects of calcium on the proliferation of lactic streptococcus bacteriophage have been studied by Potter & Nelson(50, 51, 52), who found that phage strains were unable to multiply in calcium-deficient media, although the absence of calcium did not affect the adsorption of the phage on to the host cells. Maximum proliferation occurred in calcium concentration of 0·004–0·3%, or in 2·5% calcium chloride solution used as a cell diluent. According to Collins(53) phage is unable to multiply in Niven's medium enriched with sodium acetate and Tween 80,

which will, however, support the growth of lactic streptococci; another medium with similar properties is described by Collins and his colleagues (54), which contains very little calcium and is supplemented with phosphate. It may be possible to maintain starter cultures in such media without risk of bacteriophage infection. The optimum pH for multiplication of the lactic streptococcus phage is 6·5, with a range *c.* 5·4–7·5 (55). Resistance of the phage to extremes of pH have been studied by Prouty (56) who has confirmed the original observations of Hunter & Whitehead (57) in 1940; critical pH levels for 1 min. exposure periods were 2·54–2·59 and 11·3–11·8. Thus the development of high acidity in milk cultures of lactic streptococci retards the multiplication of phage and prevents mass lysis (58). Methods for the enumeration or evaluation of bacteriophage have been described by various authors (59, 60, 61, 62); Turner & Nelson (61) use a two-layer agar-plate technique for the demonstration of plaques, while Miklik (62) uses a methylene-blue reduction test for rapid diagnosis of phage infection. Recent work on the destruction of lactic streptococcus bacteriophage has shown that sodium hypochlorite is more effective than the quaternary ammonium compounds, and a concentration of 200 p.p.m. is recommended for dairy equipment (63). Bennett (64) has substantially confirmed these observations, and has recovered *Str. cremoris* phage from the air of a room and from surfaces therein 8 hr. and 2 days respectively after spraying with infected whey filtrates; air-borne phage was inactivated by a hypochlorite aerosol giving 0·61 g. available chlorine per 1000 cu.ft. Overby (65) also recommends hypochlorite disinfection at regular intervals. Ultra-violet irradiation has been used with success by Czulak (66) in the control of air-borne phage, but the results of his experiments have not yet been published in full detail. Attempts to attenuate bacteriophage and so use it to immunize starter cultures have so far been unsuccessful (67). The term 'nascent phenomenon' has been applied to the inhibition of a strain of bacterium by a heterologous bacteriophage in the presence of a strain susceptible to that phage (68); it is believed that the sensitive strain allows the phage to multiply to a concentration at which it is able to attack the resistant strain. Collins (69) has further shown in this connexion that, even when the sensitive strain constitutes 75–95 % of such a mixed starter culture, the acid production is greater than that of the resistant strain alone; such observations have practical importance. Deane & Nelson (70) also point out that where slowness is experienced with mixed starter cultures it may be that more than one bacterial strain is undergoing lysis, or that the culture has lost its mixed character and the remaining or predominating strain is being attacked; these possibilities may represent only degrees of the same circumstance, and Graham, Nelson & Parmelee's (71) observations on the persistence of phage in commercial starter cultures have a bearing on the question. Whitehead, Hunter & Cox (72) have noted recently the adsorption of phage on to *Str. cremoris* and inhibition of its growth, without lysis of the bacterial cells or multiplication of the phage; the precise relationship between this and the 'nascent phenomenon' previously mentioned is not yet clear. Pette & Kooy (73) have described the occurrence of bacteriophages in yoghourt.

Inhibitory substances: antibiotics

The study of antibiotics has assumed vast proportions in recent years in many spheres other than that of human and animal disease, and these substances can no longer be regarded simply as therapeutic agents. Data is rapidly accumulating, for example, on their use in animal nutrition, despite the lack of information concerning their mode of action as sources of essential nutrients or as factors affecting the intestinal flora of the subject. The effects of antibiotics in milk on starter cultures have been considered in section IV (cheese), though referred to here, and the use of antibiotics for the preservation of milk is dealt with under 'Pasteurization' (p. 411).

Baron's (74) book on antibiotics reviews the literature up to early 1950; 141 antibiotics are described and a list of antibiotic-producing organisms is included. *Antibiotics. A Survey of Their Properties and Uses* (75) is the second edition of a book published in 1946 by the Pharmaceutical Press under the title of *Penicillin: Its Properties, Uses and Preparations.* Pratt & Dufrenoy (76) have reviewed the modes of action of antibiotics and trends in antibiotic chemotherapy; the

first international Symposium on chemical microbiology dealt with microbial growth and its inhibition(77). Wheater, Hirsch & Mattick(78) have described the production of an inhibitory substance by lactobacilli, which they later(79) showed to be hydrogen peroxide; the nature of the antibiotics recently described from *L. acidophilus*(80) and *L. plantarum*(81) is not yet clear. Skorodumova(82) claims antibiotic properties for certain lactose-fermenting yeasts, *M. tuberculosis* being among the species inhibited, and Hedgecock & Jones(83) have found that at concentrations of 0·05–0·25% diacetyl is capable of inhibiting the growth of many species of bacteria, particularly lactobacilli. This may be of importance so far as mixed strain starter cultures are concerned, although arginine, creatine, creatinine and dicyandiamide reversed or antagonized the action of diacetyl. The effect of ultra-violet irradiation on the sensitivity or resistance of bacteria to antibiotics is now receiving attention(84), and Rowley(85) suggests the interesting possibility of a relationship between penicillin-sensitivity and amino-acid metabolism and of its genetic control.

The early work on the antibiotic nisin was covered in the last review of this series(86). Hirsch and his colleagues have continued their work on nisin and have found important practical applications for it(87); the inclusion of a nisin-producing strain of lactic streptococcus in cheese starters prevented gas distension or 'blowing' of Swiss-type cheese due to clostridia. In a survey of streptococci Hirsch & Wheater(88) found no antibiotic-producing strains other than *Str. lactis* and *Str. cremoris*; they found, however, a lytic enzyme, produced by group N streptococci, for which they have proposed the name 'streptozyme'(89); single strains of *Str. lactis* also appeared to be capable of producing more than one antibiotic(90), and the lactic streptococci and their antibiotics could be differentiated by serological typing (see also (14)), and by cross-test and resistant-strain tests(91). A British Patent(92) covers growth media and extraction methods for improving the yield of nisin from *Str. lactis*. Wilharm & Ciblis(93) point out the need for careful selection of mixed starter strains in view of the mutual antagonism of *Str. lactis* and *Str. cremoris*. Baribo & Foster(94, 95) describe a growth inhibitor (of *L. casei*) produced by lactic streptococci; studies of this substance are not yet complete, but so far its properties closely resemble those of nisin. Good results with nisin against *Str. uberis* infections of the udder have been reported by Hulse & Lancaster(96).

The effects of residual antibiotics in milk raise serious problems in the dairy industry, particularly in cheese manufacture; Claybaugh & Nelson(97) have reviewed the occurrence and effects of antibiotics in milk. Jepsen & Overby(98) have considered various methods, including the use of chemicals, for the elimination or inactivation of penicillin in milk, and were successful in developing a resistant strain of starter culture which would tolerate 1 unit of penicillin per ml. of milk; however, this strain lost its power to form aromatic compounds and was therefore unsatisfactory. Trembath(99) advocates the introduction of penicillin-resistant starter cultures. In a review, Czekalowski(100) has covered the occurrence and development of penicillin-resistant strains of bacteria; with Jepsen & Overby, he(101) has obtained good results with bacterial penicillinase. It has also been shown that residual sulphonamides in milk may have effects similar to those of residual antibiotics(102). In Sweden it is now compulsory for veterinary surgeons to report to the dairy, penicillin treatment of cows whose milk is to be processed there, and 3 days must elapse after treatment before the milk is used for manufactured products(103); Overby(104) suggests a time interval of 2 days for chloromycetin, 3 days for streptomycin or terramycin and 4 days for aureomycin. When penicillin in oil is used, the antibiotic can still be detected in the milk 8 days after treatment(105). Despite wide variations in his results, Jørgensen(106) maintains that the amount of antibiotic present at the first milking after infusion is related to the amount originally injected. Inhibition of starter organisms by disinfectants, particularly quaternary ammonium compounds, in milk has also been described(107, 108, 109, 110).

Numerous tests are employed for the detection of antibiotics in milk, techniques being based variously on acid production by starter cultures(111), methylene blue(112), phosphatase(113, 114), the inhibition of *B. subtilis*(115), *L. bulgaricus*(116) and *Str. agalactiae*(117, 118), and resazurin(119); the last three references concern primarily the assay of nisin. A very full paper by Silverman &

Kosikowsky (120) covers the systematic large-scale testing of milk for the presence of inhibitory substances; a disk assay method with *B. subtilis*, detecting 0·3 unit/ml., is used for antibiotics, the colour reaction test, detecting a concentration of 0·005% for sulpha drugs, while the Miller & Elliker test (108) for quaternary ammonium compounds detected 3 p.p.m. in milk.

It is well known that raw milk possesses antibacterial properties, and the name 'lactenin' was given by Jones & Simms (121) in 1930 to the substance responsible. Wilson & Rosenblum (122) have now shown that lactenin is bactericidal rather than bacteriostatic; they have also surveyed its activity against streptococci of the different serological groups and found that it had a marked effect only on group A streptococci. A bactericidal substance specific for non-faecal coliform organisms has been described in raw milk by Morris & Edwards (123); the substance was also distinguished by its low temperature of inactivation, viz. 55° C. for 30 min. According to McEwen & White (124) the antibacterial properties of milk do not vary with season or diet, or even when mastitis infection is present, but weaken during the drying-off period. Further contributions to the knowledge of lactenin have been made by Auclair, Berridge and Hirsch (125, 126, 127, 128), who have separated it into two distinct substances designated L_1 and L_2.

III. BUTTER

Manufacture

Flavour and aroma are important factors in determining the quality of ripened cream butter and these in turn depend on the ability of the butter culture to promote the formation of diacetyl in butter. It has been found that, in practice, the aroma (creatin) test on a starter did not always accurately predict the quality of the butter, and ter Veer (1) suggested that the creatin test on the butter serum would be a more reliable indication of the possible diacetyl content of the butter. A negative test on the butter serum would give warning of deterioration of the starter. Evenhuis (2, 3) then suggested that a measurement of the citric acid content of the ripened starter was a better indication of its suitability for buttermaking. If the bacteria constituting the starter destroyed the citric acid too quickly there would probably be insufficient in the ripe cream at churning to ensure the development of diacetyl in the butter. Too much residual citric acid, on the other hand, would indicate that the aroma bacteria were inactive or the inoculum too small. A negative citric acid test in the starter or cream at churning would suggest that the inoculum had been too large or the culture incubated at too high a temperature or for too long a period, and adjustments could be made accordingly. Evenhuis, Lerk & Brouwer (4, 5, 6) studied the reactions by which diacetyl is produced, and from their researches it appears that the reaction is an oxidation-reduction process, that citric acid is directly concerned, that pyruvic acid is an intermediate product, that, concurrently with the diacetyl, a non-volatile substance reacting similarly with hydroxylamine and ferrous salts is also produced from pyruvic acid and that the final oxidation to diacetyl is a bacterial and not purely chemical oxidation—it cannot be induced by shaking cultures in which the bacteria have been destroyed by heat, acid, or disinfectants. Evenhuis *et al.* (5, 7, 8) advocate that, for the manufacture of butter with a good diacetyl aroma, the starter should be so managed that the cream still contains citric acid at the time of churning. The small residuum of citric acid in freshly made butter quickly disappears, but diacetyl formation from pyruvic acid may continue for several hours. Too thorough washing of the butter is not recommended. Camus, Laniesse & Burdin (9) and Doležálek (10) have also noted the influence of the size of inoculum and temperature and period of incubation on the diacetyl content of starters. The maximum amount developed in 18 hr. at 25° C. (9) and 24 hr. at 23° C. (10), but there was a decrease on continued incubation. Doležálek also observed diacetyl production in cultures of *Str. lactis*, *Str. cremoris*, *Str. thermophilus*, *L. bulgaricus* and yoghourt.

Bacteriological quality

Surface taint, a widespread defect of butter, has been the subject of much research which Wagenaar (11) has reviewed in detail. The causal organism is difficult to cultivate, and since it

does not grow vigorously on test media no quick but certain method for its detection in control work has been developed. A reliable estimate of the number of yeasts in ripened-cream butter is also of value, but Holwerda (12) found that only a proportion of the yeasts would grow on the medium in general use (dextrose yeast-extract agar, pH 3·5–3·6), at 22° C., while acidophilic bacteria would also grow at this acidity. Adjustment of the medium to pH 4·0–4·1 gave more reliable figures. The yeast and bacterial colonies were generally distinguishable and selective counts could be made. Järvik (13) has described acetic acid-producing bacteria (Gram-negative rods or vibrios, with optimum growth at 25–30° C. and pH 3·5–4·2), which were found in large numbers together with yeasts in acid butter in which a typical off-flavour had developed. These bacteria oxidized alcohol to acetic acid.

The so-called 'yeasty' flavour in butter may be due to yeasts or bacteria or a mixture of both. Bertelsen (14) found that yeasts were dominant and the only source of the taint in 60% of the unsalted, acid butter samples, but the flora of neutralized butter was more varied and in only 16% of these samples were yeasts the sole cause; bacteria also were implicated. Persson (15, 16) has connected fluctuating and seasonal incidence of yeasty flavour in butter from certain districts in Sweden with air-borne yeast infection from fruit-trees and bushes around the creameries. Weather records for 2 years indicated a close relationship between proximity of orchards, strength and direction of winds, and incidence of yeasty butter in the Skåne and Stockholm areas. Microbiological studies by Kelly & Pady (17) of the air over some arctic and non-arctic regions of Canada illustrate how heavily the air may be infected at times with micro-organisms which must have been carried from distant areas in air currents.

In Italy, Negretti (18) made butter, on laboratory and industrial scale, from milk treated with H_2O_2 and, 8 hr. later, with catalase to remove excess H_2O_2. The yield and flavour were unaffected but the butter made from the treated milk contained fewer bacteria and was of better keeping quality. In Russia, Bogdanov (19) found that the addition of yeasts to fresh sweet-cream butter restricted the growth of proteolytic bacteria and improved the keeping quality.

Stale rancid cream will seriously affect the quality of butter made from it, and the measurement of the water-insoluble acids (W.I.A.) is used to indicate the degree of lipolysis and hence its suitability for buttermaking. Hillig (20) has devised a rapid modification of the official W.I.A. test which is sufficiently accurate for use as a 'sorting' test. The acids are dissolved in ether or benzene and titrated. Purko, Nelson & Wood (21) found that the amount of W.I.A. liberated by *Geotrichum candidum* (*Oidium lactis*) was greater than for all other lipolytic organisms in cream with the exception of *Candida lipolytica*. The rate of lipolysis was depressed by rising acidity; nevertheless, considerable amounts of W.I.A. were still liberated when the cream contained *Str. lactis* or *L. bulgaricus*. In the manufacture of butter by the 'continuous process', some of the water-insoluble acids and about one-third of the butyric acid are removed in the centrifuge but there is little or no loss subsequently. Thus though the W.I.A. content of continuous process butter does not materially differ from that of churned butter the butyric acid content is higher (22). The W.I.A. content of continuous process butter did not change during 4 months of cold storage (20).

Storage

Economic conditions necessitate the storage of surplus summer butter to augment winter supplies and often import from distant lands to supplement home-produced supplies. Thus considerable quantities of butter are consumed, not in the fresh state, but after deep-cold storage, often for several months. From the data accumulated during 10 years of butter grading, Keestra (23) was able to show that the quality of Dutch butter deteriorated but little during 2 months of storage, but with longer periods typical defects, such as oiliness, began to appear. Butter from some factories, however, retained its good quality for 8 months.

A problem of great importance is the prevention of surface mould growth which detracts from the appearance and often from the flavour of the butter so affected. This has recently been studied in some detail in Sweden by Thomé & Olsson (24), and Jensen, Pedersen & Overgaard (25) in Denmark. These researches were on similar lines and brought similar conclusions. Two

favouring conditions were free moisture and low NaCl content in the butter. Infection of the butter itself with mould spores did not inevitably result in surface growth; surface infection during the printing and wrapping was a greater danger. Moisture was an important predisposing factor; humid store rooms, wet and unseasoned timber casks, scalded, wet-salted or otherwise wetted casks, wet brine-soaked parchment linings all accentuated the danger of surface mould growth. The distribution of this cold-stored butter presents yet another problem. If, as is now the accepted practice, it is packed in accurately measured ½ lb. or ½ kg. prints the large bulks must be thawed and re-worked before the prints can be made, with the accompanying danger of infection from the air and plant. In Sweden summer-produced butter is neutralized and stored for use during the winter, when it is re-worked and packaged for distribution in ½ kg. prints. Svedberg(26) showed that it was not a good practice to blend fresh (acid) and stored (neutral) butter; the keeping quality of the blend was less than that of either constituent. There was always more free moisture in the finished print than in the original bulk, a condition favouring bacterial increase. Bergman & Joost(27) found that butter would maintain its quality during cold-storage equally well in the form of ½ kg. prints or as 25 kg. lumps. If, however, the stored butter was later formed into prints these deteriorated in quality more rapidly than the corresponding prints made immediately after churning. The serum droplets were larger and proteolytic bacteria and yeasts increased, while the counts in the original prints decreased. There was a definite consumer preference for the prints which had not been re-worked. In yet another series of similar experiments Bergman, Svedberg & Joost(28) avoided this difference in keeping quality between the re-worked and the original prints by re-working the cold-stored butter in the same rollerless churns and continuing the working without the addition of salt or water until the butter was 'dry' and the moisture dispersed in fine droplets as in the original prints. No significant physical or chemical differences (bacteriological tests were not recorded) were then observed between the two series of butter prints. In England only a small proportion of the butter is home-produced and large supplies of acid and sweet-cream butter are imported often from distant countries. Most of the butter is cold-stored for long periods, and must be re-worked before distribution. Hiscox, House and Cookson(29, 30) have shown that while there was little or no increase in the bacterial count on incubation of samples as taken from the cold-store, large increases occurred when these were re-worked, either in the laboratory or at a commercial plant, especially when blends were made of acid and sweet-cream butter. A high proportion of large droplets could be detected in these samples.

It would appear that the re-working of cold-stored butter may result in loss of quality unless it is continued until the stage is reached at which the droplet dispersion is as fine as in freshly made butter. There is evidence(26, 27, 28) that pre-packed prints are just as suitable for cold-storage as larger (25 kg.) bulks of butter. A closely sealed wrapper is essential for these prints. Lined aluminium foil was well suited for this purpose; the butter retained its moisture and its colour(28, 31, 32).

IV. CHEESE

Various types

Cheese of the Cheddar and Emmental types have recently been the subject of various studies, but others, less well known, are also described. Various Italian-type cheese are increasingly consumed in America, and their typical flora and ripening processes are being examined to establish suitable methods of manufacture. Bacteria appear to play a minor role in the ripening of Provolone cheese(1, 2); rennet enzymes seem to be the chief factors. Cheese made with rennet extracts and purified rennet pastes lacked the characteristic flavour found in those in which crude pastes, especially those prepared from the stomachs of young goats, were used. The rennet pastes contained few bacteria and these were not of the types usually regarded as important in cheese ripening. They were, however, rich in lipases. The flavour of Romano(2, 3) cheese also depends on lipase activity. Mozarella, a sour-milk cheese, originally made from raw milk, lacked the characteristic flavour when made in America from pasteurized milk. Kosikowsky(4) found that the use of commercial starters (*Str. lactis*), even in small amounts, gave acid, over-ripe cheese

of dry texture, but when *Str. faecalis* was used as starter the cheese were of good acidity, with flavour and elasticity comparable with those made from raw milk. In sweet-curd Brick cheese (5) *Str. lactis* predominated and reached high numbers during the first 3 days, then rapidly decreased. Micrococci and enterococci then gained the ascendancy, but their numbers were never high and they appeared to play little part in the ripening process. De Vleeschauwer, Moulaert & Hendrickx (6) have made extensive studies of Saint-Paulin cheese, and in these also the bacterial count decreased steadily during ripening. The sugar had usually disappeared before the cheese were salted. Experiments by Zeilinger (7) indicate that yoghourt cultures could be successfully used as starter cultures in the manufacture of Romadur cheese.

Cheddar

The constituent bacteria of a starter may profoundly influence the ripening processes of a cheese. Deane (8) found that the addition of a pure culture of acido-proteolytic micrococci to a normal commercial starter did not affect the soluble nitrogen and volatile fatty acid content. If, however, *Str. liquefaciens* was added to the starter the cheese were bitter and unpalatable. The use of a mixed culture of *Str. lactis* and *Leuc. citrovorum*, or of a commercial starter containing *Leuc. citrovorum* induces gassiness in Cheddar cheese (9).

The relative importance of bacterial and rennet enzymes in the ripening of Cheddar cheese has been examined by Zimmerman (10), who compared the proteolytic activity of rennets and cell-free extracts of *Str. lactis*, and by Baribo & Foster (11), who compared the enzymes extracted from disrupted cells of *Str. lactis*, *L. casei* and *M. freudenreichii* with an enzyme extract from a year-old Cheddar cheese. In each case it was evident that enzymes of both types were concerned. In this connexion reference may be made to the finding of Peltola & Antila (12) that the addition to the curd, at hooping, of about 3 times the amount of rennet used in the initial coagulation hastened the ripening and improved the flavour of cheese. It is known that bacterial enzymes are not destroyed with the death of the organism but may continue their activities after their liberation from the autolysed cells. In discussing the heat stability of the decarboxylase system of *Str. lactis* and *Str. diacetilactis*, Oliver (13) suggests the possibility of a similar stability in other enzymes of other bacteria, and speculates on the effect on cheese ripening of the enzymes of bacteria destroyed by heat treatment of the cheese milk, and hence to what extent pasteurization can render a raw milk of high bacterial content suitable for cheesemaking.

The separation of a mixture of reducing sugars is not readily effected by simple chemical analysis, and until recently little definite information was available concerning the changes in the sugar content of ripening cheese. Fagen, Stine & Hussong (14) used paper chromatography to identify the sugars in Cheddar cheese. Very little lactose remained when the cheese were put to press, nevertheless traces could be found until 12 days later. Galactose was not present in appreciable quantities until after cheddaring, but it persisted for 19 days in raw milk cheese and for 39 days in the pasteurized milk cheese, which suggests that many of the galactose-fermenting bacteria were destroyed during pasteurization. Glucose, the most readily fermented sugar, was not detected at any stage, it was probably used as quickly as it was produced. With increasing concentration of NaCl in the cheese, the lactose and galactose persisted for longer periods.

Blue-veined cheese

In many respects the ripening of Stilton cheese (15) during the preliminary white stage proceeds much as in a Cheddar of the same age. The cheese are, however, more acid, contain more moisture, and, because of their high initial acidity, are more heavily depleted of calcium and phosphorus. With the growth of the mould there is a significant increase in the amount of soluble nitrogen, ammonia, and water-insoluble volatile fatty acids. The characteristic coat is formed by successive growths of bacteria, yeasts and moulds, but the flora has not been examined in sufficient detail to allow comparison with the coat of Minnesota blue cheese (Roquefort type) described by Morris, Combs & Coulter (16). This is slimy at first, later reddish orange, sticky and putrid, and formed by a growth of yeasts succeeded by rod-shaped bacteria and a few micrococci.

Though it is probable that the external ripening due to the smear flora affected the quality of the cheese, it could not be demonstrated with certainty.

In the manufacture of Roquefort and Blue cheese it is usual to infect the cheese milk or sprinkle the curd with dry mould spores. Sjöström & Malm(17) experimented with the use of actively growing cultures. The mould culture was beaten to break the mycelium into small fragments before it was added to the milk. Cheeses made in this way gave indications of an earlier start to mould growth, but after 3 months' ripening there was little difference between the casein- and fat-breakdown, flavour and aroma of these and the control cheese inoculated with spores in the normal way. Knight, Mohr & Frazier(18) induced the development of white mutants of *Penicillium roqueforti* by means of ultra-violet irradiation. Morris, Jezeski & Combs(19) have used these white mutants in the manufacture of Roquefort cheese. The flavour was similar to, but somewhat milder than, that of the cheese made with blue strains.

Swiss type

These cheese are peculiarly subject to butyric acid fermentation, a defect which causes great loss. Much of the research during the past 2 years has been directed to the examination of the underlying causes and their prevention. Silage is known to be a prolific source of spores infecting cheese milk, but it is a valuable foodstuff, and as such is increasingly used as cattle fodder. Thomé, Swartling, Lodin, Mattson, Wessner, Buhrgard & Lindgren(20) have made detailed experiments to study the extent to which this practice at the farm may affect the quality of Swedish cheese. They found that 'blowing' rarely occurred in cheese made from milk containing <10 spores per 1 ml. but if the milk contained 100 spores per 1 ml. about 50% of the cheese were blown. At the farms where no silage was fed the spore content of the milk was low and this was, in general, also the case when silage of good quality was fed. When the silage was heavily infected with spores, the spore content of the milk usually was high, though the infection could be reduced by careful methods of milking. The butyric acid content of spore-infected silage was high, but the absence of butyric acid fermentation did not necessarily imply freedom from spores. If the raw material of the silage was spore-infected, these spores might remain viable throughout the ensilage, although conditions in the silo were not favourable for germination and multiplication. Lind(21, 22), in Denmark, arrived at similar conclusions. Whilst there was little danger in feeding correctly processed silage, the spore content of the dung of animals fed spore-infected silage was high and therefore a potential danger to the milk.

It is a common practice to add substances such as $NaNO_3$ to cheese of this type to suppress butyric acid fermentation. Tests by Thurell & Sjöström(23) indicated that $NaNO_2$ was more effective, disappeared from the cheese at an earlier stage and showed less tendency to produce off-flavours and discolorations. Recently, interest has been shown in the use of nisin, an antibiotic produced by certain strains of *Str. lactis*. Hirsch, Grinsted, Chapman & Mattick(24) were able, by adding a nisin-producing strain of *Str. lactis* to the normal starter, to suppress butyric acid fermentations in Swiss-type cheese made from milk heavily infected with spores of a gas-producing strain of *Clostridium*. All the cheese in which the normal starter alone had been used were blown within 22 days. Similarly, McClintock, Serres, Marzolf, Hirsch & Mocquot(25) suppressed, or at least delayed, blowing in spore-infected processed Gruyère cheese by adding a solution of nisin, or a nisin-producing strain of *Str. lactis* at the time of processing. As a preliminary to cheesemaking experiments Kooy & Pette(26, 27, 28) examined 350 cultures of streptococci isolated from milk, cheese and faeces and found only 11 that produced antibiotics active against other streptococci. These strains were also inhibitory to certain anaerobic spore-bearers. The use of the antibiotic-producing strains of streptococci as starter generally prevented butyric acid fermentation, but in one of the experimental cheese the fermentation was delayed but not suppressed, and no antibiotic could be detected. This cheese contained a strain of *L. plantarum* capable of destroying the antibiotic produced by the starter and which could, moreover, frequently be isolated from raw milk and raw-milk cheese. With the exception of this one trait, it showed the normal characters of *L. plantarum*. On the other hand, Hirsch, McClintock &

Mocquot(29) isolated from 24 hr. old Gruyère cheese lactobacilli which were themselves inhibitory to *Cl. butyricum*. The inhibitory substance produced differed from nisin in that it was heat- and acid-labile, and was later shown to be a peroxide.

Mueller, Nelson & Wood(30) did not find that the presence of copper in low concentration greatly affected the growth of the organisms of Swiss cheese. *Str. thermophilus* was slightly retarded at concentrations of 16–24 p.p.m.; lactobacilli were sometimes stimulated at low concentrations but retarded at higher. Swiss cheese containing not more than 8 p.p.m. copper ripened normally with volatile acidity, flavour, eye formation and texture similar to that of the controls. With 18 p.p.m., the cheese were of poor quality; the total volatile acidity was low but the ratio acetic/propionic was normal. Runov(31, 32) found that the extracts from some Soviet Swiss-type cheese were capable of lysing lactobacilli. The streptococcal bacteriophage in filtrates from Yaroslavl cheese was reduced or destroyed by many of the lactobacilli and also by *P. roqueforti*, but few of the cheese streptococci possessed this power.

Flavour and aroma

The nature of the flavour substances, the chemical reactions on which they depend, and the extent to which variations in the flora are responsible for the characteristic flavour of the corresponding type of cheese is still largely a matter for speculation. Broad lines of differentiation are well known, and there have been advances in the knowledge of finer distinctions between them and their significance. Microbiological assay and partition chromatography are increasingly used for the separation, identification and estimation of closely related break-down products of the various constituents of the cheese. Paper chromatography, giving qualitative or roughly quantitative results, has been the more general method.

The *volatile fatty acids* of a wide range of cheese have been examined and from the publications of Krett, LeBoyer, Daume & Stine(33), Storgårds & Lindqvist(34), Harper & Gould(1,2,35), Hiscox, Harrison & Wolf(15) general deductions may be drawn as to the characteristic patterns of several well-known types of cheese. Normally Cheddar contains acetic acid but neither propionic nor butyric. Traces of the water-insoluble fatty acids may also occur, but fat lipolysis is limited in the modern quick-ripening mild-flavoured type made from pasteurized milk. Svecia, Gouda, Port du Salut and Herrgård cheese also contain only acetic acid, but propionic or butyric may develop in abnormally ripening Herrgård.

Mature Emmental and other Swiss-type cheese contain both acetic and propionic acids; acetic acid is produced in the early stages, but propionic acid quickly develops with the eye-formation. Butyric acid is characteristic of those cheese which have been infected with spore-bearing anaerobes such as *Cl. butyricum*.

Fat lipolysis is an important factor in the ripening of blue-veined cheese and a wider range of volatile acids develops. Stilton, Roquefort and Danish Blue contain acetic, butyric, caproic, caprylic, and appreciable quantities of water-insoluble fatty acids. In some circumstances valeric (or iso-valeric) acid also develops. Shipe(36) has shown that the lipase of *P. roqueforti* hydrolyses tributyrin, tricaproin and tricaprylin in that order, and that the relative specificity (e.g. 3:1 for tributyrin:tricaprylin), is the same for single triglycerides or mixtures. The hydrolysis of tributyrin was but little affected by the presence of butyric, oleic or caprylic acids. The reaction was at an optimum at pH 5·0–5·5. These data were obtained by experiments on laboratory cultures, but if a similar sensitivity exists in cheese it might help to explain the relative proportions of the acids in the mould-ripened cheese.

Rennet pastes appear to be essential to the correct ripening of Provolone and Romano cheese, and their lipases are probably more active than the bacterial flora in initiating the chemical reactions involved. Considerable quantities of butyric acid develop and this acid seems to be more closely related to the quality of Provolone cheese than the acetic and propionic acids produced at the same time.

Amino-acid patterns for a wide range of cheese are accumulating, with the help of microbiological assay and partition chromatography. Kosikowsky(37) and Block(38) have studied

Cheddar cheese, Simonart & Mayaudon(39) Dutch and French hard, blue-veined and soft-types, Fagen & Hussong(40) thirteen types including Cheddar, Emmental, Roquefort, Camembert, Storgårds & Lindqvist(41) Herrgård, Svecia, Emmental, Camembert, Roquefort and others. All the amino-acids of casein have been found in the free state in these cheese; generally each cheese contains the whole range. It is doubtful whether the specific flavour of any type of cheese can be linked with the presence of any specific amino-acid or indeed with any specific pattern. Some points, however, stand out clearly. The amino-acids are not necessarily present in the cheese in the same proportions as in casein. For instance, as Storgårds points out, methionine and threonine, which are present in relatively small quantities in casein, are relatively abundant in cheese, and while proline and valine are present in almost equal quantities in casein, valine is always found in appreciable amounts in cheese while proline is frequently absent. The presence in many ripe cheese of asparagin, glutamin, tyramin and α-amino butyric acid suggests that further decomposition of the amino-acids may occur. He refers to the ability of the propionic acid bacteria and *Str. faecalis* to break down various amino-acids including arginine. In collaboration with Lindqvist, Storgårds(42) has used electrophoresis of cheese extracts to study the nitrogen breakdown products intermediate to paracasein and the amino acids. This method offers the possibility of new sources of information, for the diagrams of the cheese types Herrgård, Roquefort and Stilton, Port du Salut, Camembert are distinct and characteristic.

Mulder(43) has emphasized the importance of continuing the studies of the flavour substances of cheese, and the urgency of restoring to cheese the characteristic and appetizing flavours which modern methods of manufacture threaten to abolish. Way(44) reports that in the Toronto area, Canada, there is a growing preference for mild cheese especially amongst the young. Preference for old cheese increased with the age of the consumer.

Defects

A recent outbreak of rusty spot in Swedish cheese appears to be similar in nature to that which has been reported from time to time for other types of hard cheese. The causal organism, isolated and described by Thomé & Lindgren(45), closely resembled *L. plantarum* var. *rudensis*. As has been found with other varieties subject to this discoloration, the organism did not always produce the pigment and could be isolated in large numbers from cheese showing no sign of discoloration. The presence of lactose and a low Eh. in the cheese favoured this defect, and the authors suggest that slow starter development and the presence of bacteria such as coliforms, which may cause a rapid fall in the Eh., may be contributing factors. A red discoloration of a different nature in Camembert cheese has been shown by Jacquet & Saingt(46) to be related to the presence of nitrate-reducing micrococci in cheese of an exceptionally high nitrate content. The nitrites thus formed reacted with protein degradation products to produce a coloured nitroso compound. *Ps. fragi*, and *Ps. viscosa*, which may gain access to cottage cheese through the washing water and imperfectly cleansed equipment, may give rise to slimy and gelatinous curd(47) or, since they are capable of destroying diacetyl, be responsible for the loss of the desirable flavour(48).

Hood & Smith(49) have suggested that gas production in processed cheese, due to the activities of clostridia, could be controlled commercially by ensuring NaCl content of 4·0% and pH 5·2 in the cheese emulsion. Other workers have found the addition of nisin or cultures of antibiotic-producing strains of *Str. lactis* to be successful.

Antibiotics and disinfectants

With the increasing use of antibiotics in the treatment of bovine diseases and of quaternary ammonium compounds for the cleaning of dairy equipment new problems have arisen for the cheesemaker. These substances are inimical to the growth of many bacteria, amongst them the lactic acid streptococci, and their presence in milk may render it unfit for cheesemaking. Penicillin is the antibiotic most generally used for the treatment of mastitis in cows, and it has

been the subject of several studies. The sensitivity of various bacteria towards it varies. Jensen & Pedersen(50) have shown that *Str. thermophilus*, the most sensitive, was appreciably affected by as little as 0·01 i.u./ml., *Leuc. citrovorum* and *Str. cremoris* by 0·05 i.u./ml., *Str. lactis* by 0·1 i.u./ml. and *L. helveticus* by 0·2 i.u./ml. Propionic acid bacteria, and butyric acid bacteria were more resistant. Dumais(51) also reports that a cheese starter was slightly inhibited by 0·1 i.u./ml. Wilkowske & Krienke(52) found that *L. bulgaricus*, *L. acidophilus* and *L. casei* were slightly inhibited by 0·3–0·6 unit/ml. Jacquet, Delacroix & Gondouin(53) state that 0·15 unit/ml. in the bulk milk caused spoilage of Camembert cheese.

The amount of penicillin in milk is greatest at the first milking after treatment and decreases progressively, but effective quantities may still remain up to 3 or even 5 days according to the preparation used and the dose administered. Various methods have been suggested to circumvent the loss this may mean to the cheesemaker, and these are discussed by Jensen & Pedersen(50), Scott(54), Auclair(55), Jepsen & Overby(56), and Storgårds(57). Diverting the milk to purposes other than cheesemaking is an obvious method, but in practice this is difficult to control, and unsuitable milk may still reach the cheese factory. As little as 1% of milk taken 12 hr. after treatment might be sufficient to render an otherwise normal bulk unfit for cheesemaking. It is sometimes possible by prolonged culture to produce starters with increased resistance to penicillin, but often the efforts do not succeed. Jepsen & Overby obtained, after forty transfers, a starter which had adapted itself to a concentration of 1·0 i.u./ml. in the milk, but meanwhile the more sensitive leuconostocs, and with them diacetyl production, had been lost. Penicillin may, however, be inactivated by penicillinase, an enzyme produced by certain bacteria including *B. subtilis* and some coliform organisms. It is advisable to add the penicillinase to the milk at least 1 hr. before the starter is added. It is suggested that the addition of penicillinase should be a routine in cheese factories, but its high cost has hitherto made this prohibitive. Penicillinase is, however, being produced on a large scale and more cheaply in Denmark. Cheese made from penicillinase-treated milk ripens normally and no flavour is imparted by the penicillinase.

Antibiotics other than penicillin may sometimes be administered, and since most are more inhibitory than penicillin towards the lactic acid bacteria their presence in milk is an even greater danger. Bell, Flora, Reaves & Holdaway(58) found that the milk of cows receiving intramammary infusions of 200 mg. of aureomycin hydrochloride per quarter would not support normal starter activity until 72 hr. after the treatment. Similarly, Bradfield, Resi & Johnstone(59) found that the milk was affected until the 8th milking after treatment of the udder with aureomycin hydrochloride ointment (213 mg.). Overby(60) found that 0·01 μg./ml. of terramycin, 0·02 μg./ml. of aureomycin, 0·04 μg./ml. of streptomycin or 0·20 μg./ml. of chloromycetin in milk slightly inhibited the growth of lactic streptococci. The addition of only 2% of milk from the first milking after dosing the cow with aureomycin was sufficient to inhibit acid production in the bulk. These antibiotics were not destroyed by pasteurization at 80° C., and since no substance comparable with penicillinase is yet available for these antibiotics the author suggests that there should be no delivery of the milk at the plant during 2 days after administration of chloromycetin, 3 days after streptomycin and terramycin and 4 days after aureomycin.

If quaternary ammonium compounds are present in milk in sufficiently high concentration they also may retard or completely inhibit growth and acid production in starter and cheese. Miller & Elliker(61) found that the addition of 5 p.p.m. of alkyl dimethyl benzyl ammonium chloride slightly inhibited acid production, while the inhibition was almost complete in the presence of 25–30 p.p.m. The milling time of cheese was delayed by 15 min. when the cheese milk contained 5 p.p.m., and by 45–60 min. when 10 p.p.m. were present. Similarly, Curry & Barber(62) report that a concentration of 5 p.p.m. of quaternary retarded acid production, but it was not completely inhibited until a concentration of about 50 p.p.m. was reached. In Lindgren's(63) experiments acid and aroma production by starter cultures of *Str. cremoris* or *Str. lactis* + *Str. diacetilactis* were retarded by 10 p.p.m. of four quaternary ammonium compounds in the milk, but on repeated re-inoculation the starter organisms adapted themselves to the conditions, and acid and aroma production returned to normal. Thus, although the abnormal

reactions may soon disappear the cheesemaker would, meanwhile, be seriously inconvenienced by the sudden appearance of quaternary ammonium compounds in the milk supply.

Instances have also been cited(64, 65) of flavour spoilage in milk, cheese and butter as the result of the use of chemical weed killers on or near the grass or other crops used as fodder. It is not yet clear whether the milk is tainted directly from the weed killer or by ingestion of the crop by the cow.

Storage

The correct ripening and final appearance and quality of cheese depend to a large extent on conditions of storage. The humidity and temperature of the store-room are important factors not only in ensuring correct ripening but also in creating conditions unfavourable to the growth of mould on the surface of the cheese; treatment with anti-mould agents, waxes and oils is also widely used, the stage at which it is applied being determined by the type of cheese. Pedersen, Møller-Madsen, Birkkjaer & Jespersen(66, 67) found that daily wiping with a brine-soaked cloth was effective in preventing mould growth on the surface of Samsoe (Danish Swiss-type) cheese, but that treatment at longer intervals with well-known anti-mould agents was less successful. If these were applied in concentrations sufficiently high to be effective the rind of the cheese was spoiled and undesirable flavours developed. Treatment with chemicals was not recommended for this type of cheese. Moreover, they should not be waxed at too early a stage. The even distribution of salt from the surface toward the centre may be slow, in which case the propionic acid fermentation in the outer layers may be delayed. If the cheese were tightly sealed in wax before this stage was reached there would be danger that the CO_2 liberated, unable to escape, would accumulate beneath the rind. An intervening period of cool dry storage before waxing is desirable, and during this time the surface is rubbed with brine or oil to prevent mould growth and excessive development of slime. Bergum & Westre(68), in Norway, reached somewhat similar conclusions for Gouda cheese. When judged only on the presence or absence of mould growth early waxing of the cheese, i.e. immediately after brining, seemed the best treatment and there was no advantage in treating the cheese with Nipagin or Antim before waxing. But when the appearance of the rind was also considered, this treatment was the least successful. Nipagin and liquid paraffin containing 7% sodium benzoate controlled mould growth most effectively. Of six anti-mould agents examined by Hendrickx & de Vleeschauwer(69) in Belgium only sodium Nipagin was successful in controlling mould growth for 6–7 weeks. The use of antibiotics is specified in a U.S. patent(70) which recommends either dipping the cheese in a 4% solution of aureomycin before paraffining or incorporating the antibiotic in the paraffin. Deane & Downs(71) have shown that Parakote is a satisfactory substitute for paraffin as a wrapping for rindless natural Cheddar cheese. If the package was pressed overnight the Parakote adhered so closely to the cheese that no air-spaces remained in which moulds might grow. Aluminium foil disintegrated within a short time. The incorporation of anti-mould agents into parchment paper prevented mould development on cheese packed in it(67). A cellulose-splitting mould, *P. brevicaule*, was found to be responsible for the rotting of parchment paper in which Romadur and Limburg cheese were wrapped. The mould was isolated from the surface of the cheese(72).

Ultra-violet light is used in cheese stores to restrict the numbers of bacteria and moulds in the air. Godbersen(73) has studied the effect of such irradiation on the surface flora of two types of cheese. The growth of the surface flora of Camembert cheese was retarded, but its development was not affected in the long run, and the flavour of the cheese was not spoiled by the irradiation. The ripening of Tilsit cheese was delayed, but the ultimate quality was not affected.

Present-day conditions of food distribution make it probable that ripe cheese will be transported and stored for long periods before being consumed. The appearance and commercial value of these cheese deteriorate and much valuable food is lost if they are subjected to attack by mites. This problem has been studied in detail by Robertson(74), who describes the life history of these mites (of which there is more than one species) and the conditions that favour their growth, and emphasizes the havoc they can wreak in a very short time. Even wax coating

is no deterrent once it has been penetrated. For control she suggests thorough cleaning of the shelves in the store-rooms to remove the mites and any material on which they can feed, waxing and fumigation. A wax or wax-substitute which presents a real barrier to mites is still to be found, and fumigants may leave toxic substances in the cheese.

V. MILK CONTROL

Of the new books relating to milk control, mention should be made of Hill's *Clean Milk*(1), whose title is self-explanatory, and the second edition of Petersen's *Dairy Science: Its Principles and Practice*(2), edited by R. W. Gregory; the latter, a relatively long and all-embracing work includes good sections on milk processing, and the microbiology of milk and milk products. *Dairy Law: Sum and Substance*(3) is up to date to 30 September 1951, and deals concisely with the legislation affecting the production and distribution of milk products in Great Britain. A useful book on modern farm dairy equipment has been written by Needham(4). A series of excellent papers by Wüstenburg(5), Seelemann(6) and Schröder(7) deals with milk hygiene from the human, veterinary and social point of view. In this last connexion, American workers have made interesting observations(8) on the biochemical oxidation of dairy wastes, using a rapid chemical oxygen demand (C.O.D.) test. C.O.D. figures for *B. polymyxa*, *Saccharomyces fragilis* and a coliform organism under standard conditions are given. Of four yeasts examined, *S. fragilis* was the most active in reducing the C.O.D. of dairy waste, and the addition of small amounts of ammonium sulphate had an accelerating action.

Milk-testing standards and the legislative and technical control of milk production have been commented on by McKenzie(9) in Scotland, and Knowles(10) in Ireland; it is interesting to note the progress outlined in Knowles's paper, which reviews legislative changes over the past 25 years. Similar papers by Dahlberg & Adams(11), Coulter(12) and Bertelsen(13) apply to America, New Zealand and Sweden respectively. Chalmers & Morgan(14) have described the Scottish Milk Testing Scheme.

The problems of sterility of farm and dairy equipment, and of the production of milk of good bacteriological quality are still under attack. Hoffman(15) has shown that there is a lag period in acidity development in milk after production; the length of this period depends upon the bacterial content of the milk and thus gives an index to its keeping quality. He maintains that, with this in mind, and with careful production and handling methods, milk of good keeping quality can be obtained, without pasteurization. The Milk Commission of the National Centre in France(16) has, commendably, made the recommendation that, so far as specific tests for milk quality and grading are concerned, the particular requirements of individual dairying regions should decide which test or combination of tests is best suited to their needs. Perhaps, in view of the mass of data, sometimes contradictory, produced each year on trials and comparisons of the various tests, there is scope for the extension of such a suggestion to other countries and areas.

In 1946, the Williams Committee was appointed to examine the distribution of milk, and to advise on any changes considered necessary to ensure that clean, 'safe' milk be delivered as efficiently and cheaply as possible. The Committee's report(17) urges that pasteurization should be compulsory for all milk sold for liquid consumption, but until this can be generally applied, the sale of raw T.T. milk should be permitted. General papers on this subject include those by Mackintosh(18) and Schulz(19); Rowlands(20) has reviewed certain research work carried out by the Bacteriology Department of the National Institute for Research in Dairying. The bacterial content of milking utensils and milking machines has been investigated by many workers, among whom Kněz(21) has shown in experiments on three farms that milk obtained by properly cleaned and disinfected bucket-type milking machines had equal or lower bacterial counts than milk obtained by hand. The wet storage method of disinfection was used with 0·05% hypochlorite solution (150–175 mg. active Cl/l.). Detergents and disinfectants are dealt with in the appropriate section of this Review. (p. 404). Milone & Tiedeman(22) have made another interesting

point, contrary to general belief, in that they found no differences in bacterial count between milk stored in old, mechanically defective cans, or in new, sound ones, provided the cans were efficiently cleaned and sterilized before use; the operative factor seems to be contained in the last clause. Ellingsen(23) has published detailed figures on the bacterial content of milking utensils; he obtained counts of over 100,000 organisms/ml. from the rinses of 11·02% of milk strainers, 3·14% of milking pails, 7·69% of milk cans, 17·39% of milking machine buckets and 16·87% of teat cups examined. Less than one-quarter of his samples had bactrial counts below 100/ml. Berridge(24, 25) has studied the deterioration of milking machine rubbers due to both the oxidizing activities of *Streptomyces* and sulphur bacteria, and the absorption of fat. The tanker system of collecting market milk has been studied in America(26); although tanker milk had higher plate counts and was rejected more frequently than milk from farm vats or cans, cleaning and sterilization of the tanks appeared to be the important factor. Opinion is still divided on the relative merits of the swab(27, 28, 29) and rinse(30) methods for assessing the sterility of milking utensils and dairy equipment; Baker(31) used a nutrient broth rinse containing peptone, Yeastrel and whole milk, examining one portion immediately, and the other after overnight refrigeration followed by 24 hr. incubation. Ray(32) recommends the sterilization of dairy utensils with accessible and non-inflammable surfaces with a flame, e.g. of butane and air.

In two papers Hall(33, 34) deals with the cooling of milk, giving details of construction and methods of use of the different types of installation. Higginbottom(35) has compared the efficiency of four different coolers, but the numbers of bacteria in the milk were too low to demonstrate any differences in count due to different cooling methods. It has been shown(36), however, that a delay (of 2 hr.) in the cooling of milk after milking decreased the potency and duration of the bacteriostatic action of fresh milk. The bacteriology and testing of farm water supplies has been considered by Clegg(37), and by workers in Wales(38) who have studied, particularly, the colony count at 37° C. Coliform organisms have received much attention in this connexion, as well as in milk itself, and, although tests for coliform organisms in milk are considered later in this section (p. 401) certain work must be mentioned here. Franklin & George(39) found, in Wales, presumptive coliform counts of more than 2 per 100 ml. in 82% of water samples from dairy farms, 55% of samples from country creameries and 28% of samples from town dairies; ten or more faecal coliforms per 100 ml. were present in one-third of the dairy farm water samples examined. Wilssens & de Vleeschauwer(40) point out the danger of using the indol test alone for identifying strains of *Bact. coli* type I, since in only 53·6% of their samples containing indol-positive organisms was the presence of *Bact. coli* I confirmed by the full Imvic tests. No reference has been found to the application of serological methods in the identification of coliform organisms in water and milk. It is possible, of course, that non-pathogenic strains of colibacteria do not possess the readily distinguished 'K' antigens which Sjöstedt(41) and Briggs(42) have demonstrated in pathogenic varieties, particularly in calves. However, supposedly non-pathogenic strains of *Bact. aerogenes* have been shown by Briggs(43) to contain 'K' antigens, and Cunningham & McLeod(44) report the colonization of the bovine udder by faecal *Bact. coli*. Coliform organisms thus appear to possess a 'conditional' or 'potential' pathogenicity(43). The question is still open, therefore, but it seems probable that coliforms from sources such as milk and water may possess an antigenic pattern similar to the pathogenic varieties, whose make-up is already defined. If so, relatively simple serological techniques might prove less laborious and more productive of information on type, source and significance than the somewhat laborious biochemical tests. The serological types of colibacteria are so far by no means as numerous or complex as those of the Salmonellae. The Public Health Laboratory Service Water Sub-Committee(45) has reported on the effect of storage on coliform counts of water samples; 20–24 hr. at room or refrigerator temperature caused a considerable increase or decrease in coliform and faecal coli counts. Coliform contamination of milk is stated to occur frequently in the home(46).

A comprehensive review of human tubercular infection from milk and milk products is given

by Kröger & Möhlenkamp (47), and their paper is of particular value in that it contains interesting tables comparing the findings of various workers in different countries. Another review (48) concerns the occurrence and classification of Mycobacteria in milk; the authors also discuss the systematic position of the saprophytic acid-fast bacteria commonly occurring in milk; they recognize and give the diagnostic characters of two species, *M. phlei* and *M. lacticola*. Greve (49) urges, again, the eradication of bovine tuberculosis, and points out that, although pasteurization of milk may be a necessary protective measure, it is in reality only a palliative; in expressing this opinion, his remarks are confined, of course, to tuberculosis; the ordinary flora of milk is a separate problem.

Thermoduric organisms have received much attention, though recently they have been considered in connexion with raw milk more than in pasteurized milk: colony count methods are still favoured for these organisms. A series of papers (50, 51, 52, 53, 54, 55, 56) entitled 'Heat resistant bacteria in raw milk', covers most aspects of the occurrence, testing and significance of thermoduric organisms. The results obtained in the work described in these papers showed a significant relationship between thermoduric colony count and keeping quality, and a system of grading milk samples into four grades is suggested on the basis of this relationship; it is recommended that colony counts be obtained by incubation for 4 days at 30° C., this combination generally giving a higher thermoduric colony count than 2 days at 37° C. Two papers in the series deal mainly with the source of thermoduric bacteria, which was found to be chiefly unsatisfactorily sterilized dairy utensils, particularly milking machines and accessories (52, 53, 57, 58); the adequacy of cooling of the milk appeared to be an unimportant factor. Thermoduric organisms in raw milk were generally most abundant in summer (55, 59), and fair agreement was found between the results of methylene-blue tests and thermoduric colony counts. A second series (60, 61, 62), entitled 'Thermoduric organisms in milk', has also appeared: a full review of the relevant literature is given in Part I, and subsequently standard techniques are suggested for the laboratory pasteurization test for milk, and for rinse and swab tests of dairy equipment. Galesloot (63) has identified some of the thermoduric organisms in raw milk, and has found that *Micr. lactis* predominated. Clegg (64) has studied spore-forming thermophiles from a variety of sources including raw milk; it was necessary to incubate these cultures at 63° C. and to include serum in the Yeastrel milk agar medium. Gordon & Smith (65) have also studied aerobic spore-forming bacteria capable of growth at high temperatures, thirty-eight isolations from milk and milk products being identified as *B. stearothermophilus*, *B. coagulans*, *B. subtilis* and *B. circulans*. Little reliance could be placed on the maximum growth temperature of a strain for its species differentiation, although 55° C. appeared to be the best critical temperature in this connexion. Amended descriptions of *B. stearothermophilus* and *B. coagulans* are given, with notes on the differentiation of these and other closely related species.

At the other extreme psychrophilic bacteria have been studied by Watrous (66) and his colleagues (67). Many strains of this type failed to grow at 35° C., and some at 25° C.: it is emphasized, therefore, that incubation of test plates at 35° C. does not give an indication of the true flora of refrigerated milk. Since they are so readily destroyed by pasteurization, psychrophilic bacteria are considered to constitute good indicators of inefficient plant sanitation (66, 68). 722 psychrophilic cultures were examined by Erdman & Thornton (69), and were identified as species of *Pseudomonas*, lactobacilli, streptococci, *Bacterium* (*aerogenes* and *coli*), *Flavobacterium* and *Alcaligenes* (one culture—*viscosus*). *Pseudomonas* species were in the majority and no spore-formers were found: all the cultures produced flavour defects in skim-milk. Aerobic spore-forming bacteria in milk have been investigated by Varma, Laxminarayana & Iya (70, 71), and were mostly *B. subtilis*, and *B. cereus* and *B. megatherium*. The heat-resistance figures which they obtained for these species, and for *B. coagulans* and *B. albolactis*, are of interest; autoclaving at 115° C./20 min. destroyed the spores of *B. subtilis* and *B. albolactis*, but spores of *B. cereus* required 115° C./30 min. or 120° C./20 min. for complete destruction.

The preservation of milk by chemical agents such as hydrogen peroxide and formalin has been dealt with under 'Other Processes' in the section on pasteurization. Three references, however,

concern specifically the preservation of milk samples prior to testing. 'Orthomercurate' tablets (composition: sodium *o*-mercurophenylcarboxylate 20%, $K_2Cr_2O_7$ 20%, NaCl 60%, fuchsin—a trace) are stated(72, 73) to have a satisfactory preservative action and to be non-toxic in character; milk samples containing this compound were kept at 90° F. for 30 days without spoilage. Orthomercurate has been used extensively in Europe and the U.S.A. Voiret(74) claims that milk samples treated with a saturated solution of salicylic acid in amyl alcohol (0·5–1 ml./200 ml.) and heated at 55–70° C. for 6–12 hr. will keep indefinitely. Clegg(75) has reviewed the literature on rejection tests for raw milk.

Direct tests

Comparisons of different stains for the direct microscopic examination of milk have been made by a number of workers; of the older methods, the North Analine and Breed techniques are preferred by Brandstein & Mundt(76, 77). A full account of the Gram stain, considered from many aspects, has been given by Bartholomew & Mittwer(78), who have shown(79) that some species of Gram-positive bacteria lose this property when exposed in smears to ultra-violet light; they point out the danger, therefore, of exposing unstained smears to sunlight. Dietze(80) found no advantage in Hucker's modification of the Gram stain, but safranin was a better counter-stain than aqueous fuchsin solution. A method of fixing, defatting and staining milk and cream films in which the smear is of uniform thickness and stains evenly has been given by Anderson, Moehring & Gunderson(81). A staining method known as the Interfacial Staining Intensification method(82) incorporates the application to the milk film, prior to methylene-blue staining, of compounds such as hydrogen peroxide, ammonium thioglycollate or the ethers of ethylene glycol; these are stated to intensify selectively the adsorptive properties of the cells in the film. The American Standard Methods methylene-blue stain has been compared with polychrome and acid-and-water-free methylene-blue stains(83, 84); the acid-and-water-free stain yielded the highest average count under standard conditions, and also showed the least variation in results(85). The distribution of bacterial clumps in standard milk films has been studied by Morgan, MacLeod, Anderson & Bliss(86), who have drawn up simple sequential tables for making rapid decisions on the acceptance or rejection of milk. The value of formaldehyde as a milk preservative has been dealt with in the section on Pasteurization (p. 412); however Levine(87) and Claiborne & Cox(88) have studied the effect of formaldehyde on the direct microscopic count of milk, and recommended it: treated milk retained approximately the same (direct microscopic) count for at least 3 days. A differential staining technique for *M. tuberculosis*, in which living cells stain blue and dead cells red, has been developed by Ehrlich(89); he has applied the technique, with some success, to cultures of *M. tuberculosis* in milk, after different heat treatments.

Zarett & Doetsch(90) have developed a medium which is selective for organisms of the genus *Proteus* and inhibitory to other organisms commonly found in milk, and Bogdanov(91) describes the preparation of nutrient media, based on pancreatin-digested skim milk, for the cultivation of lactic acid bacteria and aroma-producing, gas-producing and gelatine-liquefying organisms. A single broth medium, capable of supporting good growth of all species of lactobacilli, has been developed by Briggs(92), and has the following percentage composition: tomato juice 40·0, neopeptone 1·5, dextrose 2·0, NaCl 0·5, Tween 80 (polyoxyethylene sorbitan mono-oleate) 0·1, Yeastrel 0·6, soluble starch 0·05, distilled water to 100, pH 6·8. The addition of 2% agar to the broth forms a satisfactory solid medium.

Studies of various media for plate counts of milk have been reported by Anderson(93) and Pessin & Black(94), and it has been shown in America(95) that manipulation of the kinds and quantities of the ingredients in such media has predictable, quantitative effects on the results of the plate count. Quencer(96) maintains that incubation at 35° C. gives higher counts than at 37° C., the magnitude of the increase in count being dependent on the specific flora of the milk in question, and therefore varying considerably. In McKenzie's(97) experiments incubation at 30° C. gave a more reliable indication of the condition of the milk than 37° C. Knorr(98) has

discussed and criticized the usefulness of bacterial and coliform counts in assessing the hygienic quality of foodstuffs, including milk and ice cream; Courtney(99) has analysed data on the average plate count ratios of dairy products, and has again drawn attention to the errors arising from lack of attention to detail while carrying out plating techniques. A Petri dish cover marked with various subdivisions, for use in plate counts, has been described(100). The roll-tube method of colony count determinations has rapidly become popular in recent years: the technique is generally considered to be simpler and quicker than the plate method, and more economical of glassware, media and labour; results with the two methods show good agreement(101, 102, 103). To simplify the counting of colonies in roll tubes Tai & van Heyningen(104) project enlarged images of known sections of the bottles on to a screen by means of an epidiascope.

The colony count (and presumptive coliform test) have been recommended in Scotland for Certified, Tuberculin Tested and Standard raw milk(105), with standards of not more than 30,000 organisms per ml. for Certified, and not more than 200,000 per ml. for T.T. and Standard milk.

Although antibiotics have been considered in other places in this Review, it should be mentioned that Wilkowske & Krienke(106) have shown that a concentration of 1 unit of penicillin in raw milk was sufficient to reduce significantly the total bacterial count during 3 days' storage at 10° C., while the coliform count was not affected at penicillin concentrations of 10 units per ml.; for complete inactivation coliforms required *c.* 1000 units/ml. Opinion is still divided on the significance of coliform organisms in milk; Newman(107) has reviewed the literature on the occurrence and detection of *Bact. coli* in milk, and gives his reasons for concluding that this organism cannot alone be regarded as a reliable index of hygienic quality. In an exhaustive work, including 359 references, in which the subject of coliforms in milk is considered from many points of view, Malling(108) agrees, though more guardedly, with this opinion; of the various media which he tested eosin-methylene-blue agar was preferred(109, 110), while others prefer 'violet red-bile-agar'(111, 112). Contrary to Fournelle & Macy's(113) opinion, Thierer(114) favours sodium ricinoleate lactose broth for the detection of coliform organisms, although it is not entirely selective for these bacteria. Good results in water examinations are reported with a buffered boric acid lactose medium(115). Marçon(116) has used Zapatéro's(117) medium, which includes dulcitol with phenol red as the indicator; the medium is stated to differentiate between type I and other coliforms, but Marçon gives no experimental data, and such a test, depending on a relatively minor and variable character, must be accepted with reserve in the absence of conclusive data. Studies of the nitrate-reducing properties of coliform organisms, and of tests for coliforms based thereon, have been reported by Macris & Tzivanopoulos(118, 119), and Attimonelli(120). A simple test is described by the last-named author: a few drops of nitrate solution are added to milk which is then incubated at 37° C. and tested every ½ hr. for nitrites by the Griess reagent. Kheshgi(121) has found a general relationship between the standard plate count and the coliform count in milk, but his observations have not been confirmed by de Sackett & Gralak(122). Interesting figures showing the counts for various farm materials and utensils in India are given by Gopalkrishna & Laxminarayana(123).

The detection of tubercle bacilli in milk has been studied by several workers, of whom Paterson & Winmill(124) have used Dubos's medium with success, and Roulin(125) a modification of Ogawa's method, which incorporates a disinfectant solution to destroy the secondary milk flora. In the alcohol method described by Buss(126) 20 ml. of 36% alcohol is added to 20 ml. of milk and left to stand for 5–10 min.: a flocculate is said to result only with abnormal milk, and this is examined for the presence of *M. tuberculosis*. Melnotte, Fernier & Thomas(127) have described a centrifugation method (at 5000 r.p.m.) and Dryabina(128) a flotation technique; in the latter 50 ml. of milk are mixed with the same quantity of 0·5% KOH, and the mixture incubated at 56–60° C. for 30 min.; 0·5–1 ml. of xylol and 60–80 ml. of distilled water are then added, and the mixture is shaken and left at room temperature for 45–60 min.; the surface layer of the mixture is examined microscopically. In ninety-two milk samples examined eight positives were found by this technique, as against two by the usual centrifugal method. Techniques have

been described by Guillot, Névot & Thieulin(129) for the detection in milk of various human pathogens including the tubercle bacillus, and species of *Brucella* and *Salmonella*. 2% borax has been found to be the most suitable preservative for milk samples containing *M. tuberculosis*(130).

Indirect tests

Numerous papers concern various indirect tests, particularly dye reduction, used in milk control. Reference has been made already to the conflicting nature of much of the evidence put forward, and to the suggestion(16) that the particular requirements of individual dairying regions should dictate the test or combination of tests most suitable locally. A series of excellent papers(131, 132, 133) on keeping quality and raw milk grading have been written conjointly by workers at the National Institute for Research in Dairying, Rothamsted Experimental Station, the N.A.A.S. and the N.M.T.S.; the data showed a high correlation between the methylene-blue and resazurin tests as measures of keeping quality, but both were relatively unsatisfactory for this purpose. Instead, the clot-on-boiling (C.O.B.) test was recommended, compensation being applied for unavoidable variations in atmospheric shade temperature during storage of the samples before testing. The results showed that a standard of 21 hr. at 22° C. in summer and of 24 hr. at 22° C. in winter approximated the standard now imposed by the methylene-blue (4½–5½ hr.) test for designated milk in England and Wales. (Babad & Shenhav-Hetman's(134) figures from Israel agree closely.) An apparatus for the automatic determination (e.g. during the night) of the C.O.B. end-point is described, and Rowlands & Hosking(135) also recommend the test. The C.O.B. test (at 22° C.) has also been studied by Garvie & Rowlands(136, 137, 138) in their work on the importance of different species of bacteria in dye-reduction and keeping quality tests; they found that group N ('lactic') streptococci were usually predominant at 22° C., and streptococci, staphylococci and micrococci at 37·5° C. The differences between the flora at the two temperatures may account for discrepancies between tests made at these temperatures. Group D and group N streptococci and coliforms had the greatest effect on the keeping quality tests. Thomas and his colleagues have reported on a keeping quality test at 19–20° C.(139), and Vincent(140) has suggested a compensation scale which he claims would permit comparison of dye-reduction test results to be made at various seasons and between various countries. Clegg, Wohlgemuth, Crossley & Andrews(141) have studied the value of a variety of dye-reduction and colony count tests (thirty-six combinations in all) as indices of the hygienic efficiency of production methods; they concluded that no single test was satisfactory, and obtained the best results with a combination of the C.O.B. test and thermoduric colony count.

The methylene-blue test has been studied by many workers, some of whom have considered primarily the question of temperature compensation. Feagan, Morton & Vincent(142) give two tables representing respectively a temperature compensation scale and a calculation of mean shade temperature (see also Vincent(140)); these provide a method for the local grading of milk by the methylene-blue test equivalent to the British Ministry of Health 4½ hr. standard for accredited milk. However, as Clegg(143) and his co-workers(144) point out, no manipulation of figures can produce a satisfactory compensation scale for atmospheric temperatures above 65° F., and they suggest separate scales for winter and summer. Schönberg(145) has described a reductase test based on the ability of bacteria to reduce triphenyltetrazolium chloride (T.T.C.) to triphenylformazan, which is red. 0·5 ml. of a 1% T.T.C. solution is added to 5 ml. of milk at room temperature in diffused light; in milk with a high bacterial count the colour appears within 3–6 min., but takes 2–3 hr. to develop in milk with a low count. Following Schönberg's lead Gyllenberg(146) has also used T.T.C. as an indicator, and has found that the reaction only takes place in light, and that different organisms vary considerably in their ability to reduce T.T.C. Posthumus(147) has demonstrated the effect of hypochlorites in milk on methylene-blue reduction time, but elsewhere in this review (p. 406, Detergents and Disinfectants) work is reported showing that, when milking machines are disinfected with hypochlorites, insufficient chlorine reaches the milk to affect acid production or the methylene-blue test.

In the resazurin test, according to Roeder(148), the course of the colour changes varies widely with the temperature (18–37° C.), and the test will only detect and differentiate between milk samples with counts of < 50 million/ml., corresponding to a methylene-blue reduction time of > 1 hr. Clegg(149) has proposed a time-temperature compensation scale based on time taken to reach disk $\frac{1}{2}$, and has found such a scale to give good results in practice. The 5 min. resazurin test has been shown to be more accurate in picking out very poor quality milk than the 10 or 15 min. tests(150). Hughes & Ellison(151) have compared the 10 min. resazurin test with the alcohol test, but found little agreement: the closest association with the alcohol test was found when 76% alcohol was used. Since resazurin is very sensitive to fresh milk containing large numbers of leucocytes, it can be used on the farm as a screening test for mastitis(152), but no significant relationship exists between the resazurin and the electrical conductivity tests(153).

Comparisons of methylene-blue and resazurin tests, sometimes with other tests as well, have been many, and Lagoni(154) has found a correlation coefficient greater than 0·9 between the two tests (15 min. resazurin, taken to rose-coloured stage) with milk samples reducing methylene blue in $2\frac{1}{2}$ hr. or less. However, Angelucci(155) found no agreement between the tests. For the grading of raw milk in France, Pien(156) proposes the adoption of the methylene-blue test, in conjunction with the sediment test, but Møgensen(157) favours the resazurin test since it registers colour changes at intermediate stages of reduction and, in addition to being as effective as the methylene-blue reductase test for the grading of milk, indicates abnormalities due to pathological conditions(152, 158).

Johns(159) has studied the effect on the two tests of storage of milk samples under varying conditions; he found no significant differences with the methylene-blue test between fresh and refrigerated milk samples, but suggests, nevertheless, that samples refrigerated overnight before testing should contain the dye in the case of resazurin, but not with the methylene-blue test. Overby & Mair(160), in Denmark, have discussed dye-reduction tests and favour the 1 hr. resazurin test for dairy practice; in Australia, Trembath(161) advocates the resazurin test in preference to methylene-blue reduction for the bacteriological grading of milk on the creamery platform; this opinion is confirmed by Schubert & Nagle(162), but opposite views are expressed by Kästli & Binz(163) who consider the resazurin test unsuitable as a keeping quality test for milk in Switzerland. There is clearly great divergence of opinion still concerning the suitability of these, and other tests, for different purposes and in different countries and areas. The resolution of these problems may ultimately be achieved along the lines taken by Brogmus(164) who studied the effects of different *types* of bacteria on dye-reduction tests. The ten types considered showed a wide divergence in the rapidity with which they produced dye reduction, but for each variety of organism there was usually a measure of agreement between the methylene-blue and resazurin (and Janus green) tests. In general, resazurin gave shorter reduction times than methylene blue, and *Str. lactis* reduced quicker than *Bact. coli*. Similar work, described by Egdell & Bird(165), has confirmed these observations, and has shown that micrococci, microbacteria and *Str. thermophilus* have little influence on the methylene-blue test (at 37° C.), while Gopalakrishna & Iya(166) have confirmed the relationship between high coliform counts and short reduction times.

Defects

Knowledge of the fault known as 'broken' or 'bitty' cream in raw and pasteurized milk has been greatly enhanced by the work of Stone(167, 168) and Stone & Rowlands(169), who have shown that it is caused by the aerobic spore-formers *B. cereus* and *B. mycoides*, but not by other members of the genus, viz. *B. subtilis*, *B. megatherium*, *B. pumilus*, *B. firmus* and *B. sphaericus*. Strains of *B. cereus* which did not elaborate lechithinase failed to produce broken cream, while Stone has produced the fault with lecithinase concentrates. The major cause of the defect thus appeared to be the breakdown of the fat globule membrane by hydrolysis of lecithin. Pasteurized milk stored at 5° C. or lower did not develop the fault within 24 hr. of delivery (and consequent elevation of temperature) to the consumer. Although implicated in the spoilage of evaporated milk, and not in the above connexion, the thermal resistance of *B. subtilis* is of interest: Ruyle &

Sognefest(170) have examined a strain capable of surviving evaporated milk sterilization under commercial conditions (241° F. for 15 min.).

Rice(171) has discussed the causes and methods of prevention of the common defects in cream, and papers have appeared incriminating various organisms including *Ps. fragi*(172) and *Chromobacterium prodigiosum*(173) in milk and cream faults. Herrington(174) has reviewed the question of lipase (see (172)) in relation to flavour defects in milk and milk products, and the methods of controlling lipase production or activity. An interesting observation is made by Campion, Howard & Fischer(175), who have used the 10 min. resazurin test for examination of milk on the creamery platform; they point out the frequent occurrence of taints in milk giving disk readings in the test of 4 or higher, which suggests great bacterial activity in such tainted milks.

'Ropiness' in milk is generally considered to be due to *Bact. aerogenes*(176, 177, 178) and/or *Alk. viscosus*(179, 180). The source of these organisms varies considerably, but most authors incriminate washed milk bottles(177), the original milk supply(178, 179), or the water supply(179), while some(180, 181) go as far back as the milking equipment on the farm. As a rule infection could eventually be eliminated by treatment of utensils and apparatus with steam and hypochlorites. Claydon & Foltz(182) have described a yeast-like defect in cream giving a 'bread dough' appearance, for which a strain of *Bact. aerogenes* was responsible; they concluded, however, that the production of this defect was a variable characteristic not possessed by all strains of *Bact. aerogenes*.

VI. DETERGENTS AND DISINFECTANTS

Brauss(1) again draws attention to the sensitivity of quaternary ammonium compounds to protein which renders them unsuitable for certain purposes, while Davis(2) gives useful tables comparing the properties of detergents and disinfectants used for dairy plant and utensils. Bertelsen(3) found considerable variation in bacterial counts of ice-water and warm water in dairy plants and advocates bacteriological control of the water used, with regular inspection and cleaning of circulating systems. Despite the attention paid to chemical disinfectants, the bactericidal action of sunlight has not been overlooked; Swart-Füchtbauer & Rippel-Baldes(4) deal primarily with the correlation between light wave-length and bactericidal power, but the precise mechanism of the antibacterial action, whether inhibitory or lethal, is uncertain. Walters, Cousins & Edmunds(5) and Mohr & Jünger(6) emphasize the importance of efficient cleansing of dairy utensils prior to steam or hypochlorite sterilization, and the latter authors suggest a method of evaluating and comparing the efficiency of bottle and churn washers. Jensen & Claybaugh(7) showed that the effect of ethylenediamine tetrasodiumacetate or polyphosphates, each with wetting agents, was only slightly reduced by 10% added milk. However, with quaternary ammonium compounds Barber(8) found that the time required for destruction of *Bact. coli* ($\frac{1}{2}$ min.) was increased to 12 min., >34 min., 40–45 min. and >60 min. by the addition of 2·5% skim milk, whole milk, ice cream mix and sweetened condensed milk respectively. Similar methods of measuring the cleaning effects of detergents, using uniformly soiled milk cans treated under identical conditions, have been described by Hoy & Clegg(9) and by Mohr & Jünger(10). In another paper, Mohr & Jünger(11) show the superiority of the method of 'rolling out' a milk can with steel balls for the evaluation of detergents, over 'floating' on metal plates. The temperature of the wash water was found to be the factor controlling efficiency in mechanical can washers by Tuckey, Reinbold, Tracy & Hussong(12); at 140° F. 73% of the counts obtained were 40,000 or less, but at 160 or 175° F. 96% of the counts were under 10,000. Studies of bottle-washing under plant conditions have been made by Stark, Holland, White & Gurdian(13, 14), using sodium hydroxide, sodium hydroxide plus trisodium phosphate, and the latter mixture with tetrasodium pyrophosphate, at varying temperatures (120–170° F.) and concentrations of caustic soda (0·9–4·2%) for a standard soaking time (3·25 min.). The temperature-causticity relationship appeared to be unimportant, but tetrasodium pyrophosphate was essential to obtain satisfactorily rinsed bottles: the addition of trisodium phosphate was of no advantage,

and the optimum temperature was 160° F. No correlation was found between bottle-washing efficiency and surface tension, specific gravity or carbonate alkalinity of the different soak solutions. Baker & Goodale(15) observed no significant effect on bacterial count on omitting warm water rinses in bottle washing. A sequence of suitable rinse solutions is suggested by Moore(16) for cleaning permanent steel milk-pipelines without dismantling.

Two books, by Resuggan(17) and Lawrence(18), approach the quaternary ammonium germicides from similar points of view—chemistry, properties, antibacterial activity, etc., and each contains a chapter on the application of these substances in dairying. A comprehensive review of the literature on quaternaries, with particular reference to their use in the dairy, has also been published(19). Barber(20) describes extensive field trials of seventeen quaternary ammonium and four hypochlorite preparations, field and laboratory results agreeing closely: a reduction in the total and thermoduric colony counts of the milk from various farms followed the introduction of both types of compound, the percentage reduction figures varying between 50·3 and 65·9, and 52·7 and 90·1 for hypochlorites and Q.A.C.'s respectively. Hucker(21) records similar favourable results. The use of Q.A.C. solutions, not only for plant and utensils, but also for washing the udders of cows before milking, is recommended by Collins(22): for this purpose, warm solutions containing 140–150 p.p.m. of active disinfectant were used. Kesler, Knodt & Reid(23) claimed that hypochlorites and quaternaries were of no more value as udder washes than clean water in preventing the spread of mastitis organisms, but Hughes(24) strongly favours hypochlorite in udder washing water, particularly in the control of *Str. agalactiae* in dairy herds. Davis, Resuggan & Ive(25) obtained good results from the addition of quaternaries to the rinse water of ice-cream equipment. The subject of a British Patent(26) is the combination of alkaline inorganic, and non-ionic organic, detergents with a Q.A.C. bactericide, whereby it is claimed that cleansing and disinfection of dairy and food utensils may proceed simultaneously. Elliker, Keesling, Miller & Wilster(27) also report the successful use of detergent-sterilizer preparations, but their studies on the combination of several non-ionic detergents with a variety of Q.A.C.'s lead Dvorkovitz, Crocker & Galloway(28) to conclude that non-ionic detergents have a definitely adverse effect on the germicidal power of quaternaries. Although such combinations are practicable under certain conditions the circumstances and formulae concerned should be considered very carefully. Swartling(29), Swartling & Lindgren(30) and Lewis(31) have investigated some of the factors such as water hardness, pH and temperature, affecting the germicidal power of quaternary ammonium disinfectants, and also the effect of the presence of skim milk. Chaplin(32) found that concentrations of 260–1200 p.p.m. of a Q.A.C. formed an insoluble complex with the proteins of 10% skim milk and that the complex was stabilized by heat: for five different Q.A.C.'s the maximum loss in this way was 45–70%. Curran & Evans(33) have shown that none of eight Q.A.C.'s or two quaternary-containing detergents in concentrations up to 5% consistently killed all the spores of thirteen different varieties of bacteria in test suspensions within 30 min. at 20–60° C. The sporicidal efficiency of the substances increased with temperature and pH although decreasing considerably in the presence of skim milk. The development of specifically resistant strains seems to be a universal problem wherever antibacterial agents are employed: with quaternary ammonium compounds it is reported in five strains of *Bact. coli* by Crocker(34), and by Chaplin(35) in *Serratia marcescens*. Crocker discusses the irreversible changes induced in the biochemical and physiological properties of *Bact. coli* and their bearing on routine testing for this organism: Chaplin suggests that increased resistance in *Serr. marcescens* is due to the development of protective lipids in or around the cells, but his work requires substantiation: elsewhere(36) he has described cytological changes induced by Q.A.C.'s in *Bacillus* species. The undesirable effects on starter cultures of residual quaternaries in milk have been studied by several workers. Mueller(37) and Calbert(38, 39) have estimated that only negligible amounts of Q.A.C. solution are carried over to the milk from milking machine clusters, but Barber, Hodes & Dunne(40) and Lundstedt(41) have shown the inhibitory effect of relatively small amounts of Q.A.C. on starter cultures. Furthermore, Lundstedt has made the important observation that Q.A.C.'s were 50 times more effective against aroma-producing than against other lactic acid

bacteria. With chlorine disinfection of milking machines, insufficient reaches the milk, according to Holm & Pedersen(42), to affect acid production, or the methylene-blue test. The question of transfer or carry-over to milk of detergents and disinfectants (and antibiotics), and the resistant strain problem evidently require further examination. Jepsen & Overby(43) succeeded in producing a starter strain resistant to 1 unit/ml. of penicillin, but the strain lost the power to form aromatic compounds.

The bactericidal action of chlorine in the presence of organic matter has been studied by Cox & Whitehead(44) who showed that the chloramino compounds formed when hypochlorite was allowed to react with glycine and skim milk had a slow but definite disinfecting action against *Str. cremoris* at pH's 7·0 and 9·5. Chaplin & Johns(45) found that pH 7·2 was more favourable than pH 10·2, but that an increase in the proportion of milk present in the test reduced the effect of the neutral solution more than that of the alkaline: elsewhere Johns(46) found that a new chlorine compound, 'Antibac-25', was no more effective than existing hypochlorites against *Bact. coli*, *Staphylococcus aureus* and *Ps. pyocyanea*. Clegg & Cuthbert(47) have described a small-scale water chlorinator for farm use: Rowlands(48) has studied the use of sodium hypochlorite on small farms where hand-milking is practised, and Kästli & Binz(49) have examined five commercial disinfectant hand ointments recommended for use during milking: although accomplished in 2–5 min., the destruction of *Str. agalactiae* by these ointments was considered to take place too slowly to prevent the transmission of *Str. agalactiae* via the milker's hands. The value of calcium hypochlorite in butter factories has been investigated by Khikalo(50), who records particularly favourable results against coliforms and moulds, and states that the flavour of the butter was not affected.

Improved methods for the cleaning and sterilization of milking machines continue to be sought, and Bratlie(51) and Dahlberg, Kosikowsky, Seeley & Leventhal(52) have recommended procedures and materials for this purpose. The object of the latter authors was to devise a single detergent-disinfectant solution which would clean and sterilize machines simply by rinse washing followed by storage, and they achieved some success with detergent-quaternary combinations. Pucholtová & Nový(53) also obtained good results with a chloramine-containing detergent-sterilizer, which had no corrosive action on aluminium in 2% concentration. According to Churchill & Mallmann(54) the air-line hoses of milking machines are an insignificant source of milk contamination if the machines themselves are properly sterilized. Reid(55) sounds a note of warning in observing that on some farms where machines are used, too much reliance on chemical detergents in preference to scrubbing, and failure to replace old rubbers, was responsible for much contamination of the milk by thermoduric organisms. Hoy & Rowlands(56) have reported favourably on wet storage methods for milking machine clusters, particularly when a lye solution was used, and have found that high bacterial counts, mainly micrococci, were generally associated with old milk tubes. These observations have been confirmed by Morris & Coleman(57, 58) who obtained satisfactory results with the wet storage method even during hot weather, and by McKenzie & Lambert(59). Recommendations for the cleaning of milking machine rubbers are also made by Whittlestone(60). Mueller & Seeley(61) recommend an additional milking assembly for every two existing units, to permit a 2–6 min. immersion of each assembly in sterilizing solution between the milking of each cow.

The search for means of evaluating detergents and disinfectants continues, and Buchbinder & Zaretsky(62) have described a laboratory test particularly suitable for relatively high (over 100 p.p.m.) concentrations of the more rapid bactericides such as chlorine and quaternary compounds, based on the cessation of motility produced in a suspension of actively motile coliform organisms: results in reasonably good agreement with those of the standard Weber and Black method were obtained. Good correlation with the results of a large-scale experiment with milking machines was obtained by Goetchius & Botwright(63) in their test for quaternary detergent-disinfectants: strips of sterilized rubber were contaminated with *Str. faecalis*, *Bact. coli* and *Ps. aeruginosa*, and plate counts of these made before and after cleaning and sterilizing. A screening test for quaternary ammonium compounds based on a colony count method to

determine the survival of *Bact. coli* is used by Cousins(64). Lineham(65) has described a form of laboratory milking assembly and equipment used for the evaluation of disinfectants. Meewes(66) has investigated various techniques for official microbiological testing of detergents, and discusses the relative merits of the different methods of subsequent bacteriological examination and counting. An interesting and informative collection of papers presented at a conference on the mechanism and evaluation of antiseptics and antibiotics has been published(67). Meaney(68) describes the results of field tests of three typical dairy farm detergent-sterilizers, but the lack of detail in this paper is disappointing, particularly as it is stated that the results varied with the particular water supply on the different farms. A modification of Auerbach's method has been used for the estimation of quaternary ammonium compounds in milk by Pien, Desirant & Rochelle(69), which was also applicable to rinse water from milk cans. Miller & Elliker(70, 71) have developed a technique based on the extraction and precipitation of Q.A.C.'s in a tetrachloroethane-acetone eosin indicator solution, and they claim to be able to estimate as little as 5–100 p.p.m. of quaternary ammonium compounds in milk.

VII. PASTEURIZATION

Two books published in 1950 contain sections on milk pasteurization, butter, cheese and dairy products in general: Heiss(1) deals primarily with technology, and Tanner(2) chiefly with the relevant microbiology. Christophersen(3) has reviewed the literature on the destruction of bacteria by heat, and the influence of the different factors involved in the process. Sjöstrom(4) has discussed the pasteurization and control of market milk in Sweden, and Sa(5) the problems of pasteurization of sheep and goat milk for cheese manufacture. A case for the compulsory pasteurization of consumer milk is made by Pien(6) in France, and by Barnes(7) in Malta. Seligman(8) has made a detailed historical survey of the development of pasteurizing equpiment, and a useful article on premises and equipment for pasteurizing dairies contains the specifications and recommendations of an Australian Milk Pasteurization Committee(9). An excellent paper by Plock & Wälzholz(10) describes and illustrates the various component parts of pasteurizing plant, and measuring, control and recording instruments.

Following the trend of recent years H.T.S.T. pasteurizing has continued to increase in popularity, and there now seems to be, in the light of up-to-date information, less distrust of a pasteurization technique that dispenses with a positive holding time. Weber(11) has given a comprehensive account of the various factors which affect time and temperature in milk pasteurizing H.T.S.T. plants: he concludes that, in commercially operated plants, the actual minimum holding time will rarely be less than 15 sec., and will probably never approach the theoretical danger point of 6 sec. (i.e. at 160° F.) even under the most adverse conditions. As Lear & Foster(12) point out, it is important to determine the separate contributions to the lethal heat of preheating and holding: a direct relationship exists between the total lethal heat contributed by preheating for 1 min., and the holding temperature of the process. With holding temperatures of 146·3 and 163·7° F., 1 min. 'straight line' preheating supplied respectively 0·4 and 29·9% of the total lethal heat. Extensive experiments have been made(13) with a 3000 l./hr. plate-type pasteurizer of Austrian manufacture, as a flash pasteurizer at 81·5–87° C., or as a H.T.S.T. unit at 72·5–77° C., with 17·4–46·5 sec. holding times. Less satisfactory results against general contaminants, especially coliforms, were obtained when the machine was used in the H.T.S.T. form, but biological tests in guinea-pigs showed that pasteurization by either method of milk which had been heavily contaminated with *M. tuberculosis* or *Brucella abortus* destroyed these organisms. The coagulating properties and original protein character, but not the creaming ability, of the H.T.S.T. pasteurized milk was superior to that treated by flash-pasteurization. Stansby(14) found that holder and H.T.S.T. plants were of equal efficiency, the quality of their products being influenced considerably by the initial bacteriological quality of the raw milk. Depot milk was less satisfactory than milk direct from the farm, but the relatively good keeping quality of the pasteurized depot-milk, in spite of high counts, was probably because the flora

was composed largely of thermophiles. The effects of holder and H.T.S.T. pasteurization on milk quality have also been compared by Itzerott(15) who again found the acknowledged disadvantage of reduced cream line in H.T.S.T. treated milk, although its keeping quality was generally slightly the better; there was no difference in flavour between the two milks. Temperatures of 144–5 and 164–5° F. respectively are tentatively recommended(16) for holder and H.T.S.T. pasteurization of concentrated whole milk (one-third original volume). Information on relative temperatures and various other concentrations (of milk) would be desirable, the figures for a single point on such a curve being of limited value. It is also recommended(17) that chocolate milk should be pasteurized at time/temperature combinations of 165° F./15 sec. or 145° F./26 min.: *Micr. freudenreichii* was the test organism. Although reliance is commonly placed(18, 19) on organisms such as this, of similar heat-resistance to *M. tuberculosis*, the dangers inherent in such a practice are not defined: as Beck(20) has again pointed out, tubercle bacilli in raw milk may, under certain circumstances, survive pasteurization, possibly due to protection during heating by flocculated protein, and/or the increased cell content of the substrate; conditions not always present or even reproducible when test organisms other than *M. tuberculosis* are employed.

During the period under review detailed reports have appeared from the State Machinery Testing Station, Alnarp, Sweden, and the State Dairy Experimental Station, and Research Institute, Hillerød, Denmark, on the construction and performance of A.P.V.(21, 22), Alfa-Laval(23, 24), Silkeborg(25, 26, 27) and Kolding(28, 29, 30, 31) pasteurizers, and on a plate heat exchanger of German manufacture used as a flash pasteurizer, from the Dairy Research Institute, Kiel(32). Seelemann(33, 34) also gives detailed information on the official testing of 29 H.T.S.T. pasteurizers of different makes, and, elsewhere(35), makes recommendations for time/temperature combinations for flash and H.T.S.T. pasteurization under factory conditions. Two milk pasteurizers suitable for use in the home have been described(36, 37), and Baragar & Downs(38) have tested three such machines. Pascoe(39) has developed a high-temperature short-time laboratory pasteurizer in which average times for heating to 164° F., holding and cooling to 86° F. were 42 ± 2, 38 ± 1 and 30 ± 2 sec. respectively. These times were very similar to those for a commercial H.T.S.T. plant, and comparative colony counts of treated milk from the laboratory and commercial machines agreed well. The laboratory pasteurization apparatus described by Thomé & Olsson(40) incorporates interchangeable holder sections allowing holding times of 2·6, 29·9 or 50·5 sec.: the capacity of the apparatus is 30 l./hr., and it is claimed to permit a true reproduction of the full-scale dairy pasteurization process, and to be particularly easy to clean. Special cleaning solutions are held in built-in containers in Lindquist's(41) micropasteurizer. Despite the now rapid accumulation of data concerning time/temperature combinations in H.T.S.T. plant, information on requirements for the destruction of bacteria and phosphatase in milk are still lacking for treatment times of less than one minute. A laboratory high-temperature short-time pasteurizer, having a yield of 100 ml. of treated milk per run, is under construction at the National Institute for Research in Dairying at Shinfield (Report, 1950)(42).

Burton(43) has described a simple graphical method for estimating the effectiveness of the pasteurization obtained when the holding temperature is not steady, but the temperature-time curve is known. This is expressed as a coefficient, which is unity when bacterial destruction is equal to legal requirements for pasteurization. Inglesent & Storrow(44) suggest an adjunct to pasteurization by coating milk filter cloths or filter press plates with starch. This reduces the bacterial count of the milk and, it is stated, removes some of the coliform organisms. Additions of up to 0·5 oz./sq.ft. were satisfactory, but higher amounts reduced the fat content of the milk.

The question of control of the H.T.S.T. process is highly important and Cuttell & Ashton(45) have reviewed the position. Whitney(46) describes the installation at a large dairy of a laboratory pasteurizer with recording instruments similar to those of the main plant: this was useful for routine checking of recording and controlling instruments, and enabled faulty instruments to be tested immediately by fixing them in place of those used on the laboratory pasteurizer. In a United States Patent(47) raw milk is prevented from contaminating the pasteurized milk, if a leak develops in the heat exchanger, by keeping the latter at a higher pressure than the former.

A diaphragm operates a switch stopping the flow if the pressure gradient is reversed. Flow holding and methods of testing in the flow holders of pasteurizers have been reviewed(48): a standardized procedure for the salt conductivity test for determining holding time in H.T.S.T. plants has been recommended in America(49).

No major advance or trend in recent years has been noticed so far as tests for the efficiency of pasteurization are concerned: a useful booklet from New Zealand(50) contains an informative chapter on such tests, in which the phosphatase test is treated in some detail. Ritter(51, 52) and Mottier(53) have reviewed exhaustively the literature on the phosphatase test, and the chief contribution in this sphere has been the new phosphatase test of Aschaffenburg & Mullen(54), which is more rapid and simple than Kay and Graham's or other forms of the test(55) and has quickly achieved popularity(56, 57). However, Horwitz, Knudsen & Weiss(58) still favour the photometric Sanders-Sager method. The heat resistance of phosphatase in cream has been studied, and it was found(59) that phosphatase destruction proceeded at the same rate in milk and cream, although the latter took 0·6–0·8 sec. longer to reach the pasteurization temperature (90° C.). The same workers have also shown that yeasts and oidia from cream which they examined produced heat-resistant phosphatases. The yeast phosphatase and the oidium phosphatase required for complete destruction 15 and 32 min. respectively at 82° C. Schwarz & Lange(60) have shown that acidity reduces the phosphatase activity of milk, and have discussed the implications of this observation in the control and evaluation of efficiency of pasteurization. Hansen, Wood & Thornton(61) describe phosphatase inactivation studies in milk, and it has been shown(62) that in the phosphatase test of Aschaffenburg & Mullen the specified quantities of milk and reagents could be halved without adversely affecting the results. Periodic confirmation of the phosphatase test by the peroxidase and coliform tests is advocated by Piraux, Lacrosse & Querton(63) in an excellent general paper on pasteurization research at the State Dairy Station, Gembloux. Paschke(64) also favours a combination of peroxidase and phosphatase tests by which it was possible to differentiate between insufficiently heated milk and heated milk containing raw milk: he also considered the effect of preservatives on the tests used for evaluating pasteurization, and concluded that formalin, at the rate of 5 drops of a 33% solution per litre, did not interfere with the tests. Greater amounts reduced the sensitivity of the tests, and milk preserved with potassium dichromate could not be tested for peroxidase using guaiacol: the peroxidase test has also been reviewed by Burbelo(65). A method developed comparatively recently in Germany, the 'Lactognost'-Heyl test, has been evaluated by Schwarz & Lange(66), and Kieferle & Seuss(67). 1 ml. of milk is mixed with a warm aqueous solution of Lactognost I reagent (phenyl ester of phosphoric acid), heated to 37° C., maintained for 1 hr.: a spoonful of Lactognost II powder (a reagent sensitive to phenol in a neutral medium) is then added and the colour developed after 10 min. is measured. In H.T.S.T. plants the test detected deviations of 2–3° C. below the legal minimum, and in holder pasteurization a deviation of −1° C. The test also detected as little as 0·3% added raw milk in properly pasteurized milk, and was useful in the control of H.T.S.T. pasteurization. A test for detecting adulteration of pasteurized milk with reconstituted dried or evaporated milk is described by Edwards(68): a blue colour develops when unheated (below 75° C.) milk or cream is incubated for 5 min. at 61° C. with vanillin solution, but the activating substance was not identified. The critical temperature concerned, i.e. 75° C., of course limits the practical value of the test to the detection of adulteration, and in its present form it is hardly suitable in pasteurization control or evaluation. In a general paper(69) on faults and recontamination in pasteurization it is stated that in some cases where raw milk contamination was high, efficient pasteurization as measured by the phosphatase test did not reduce the numbers of bacteria sufficiently: further information on this point would be of interest from the point of view both of the efficiency of the phosphatase test, and the possibility of protection of pathogens by cellular matter, bacterial or otherwise, as previously mentioned (p. 408, Beck(20)). Souta & Silva(70) have shown again that post-pasteurization contamination with froth in holder plants was sometimes responsible for positive phosphatase results on the finished milk, and also for a high incidence of coliforms therein. This has been confirmed and

investigated further by Lomax & Clegg(71) who found that, in several plants which they examined, the froth was 4–6 in. deep and 7–10° F. below the temperature of the milk itself. Conditions were satisfactory in other types of holders where the vats were filled almost to the brim, and there was very little air space and no froth.

So far as the cleaning of pasteurizing plant is concerned, Botham(72) suggests the following procedure: a 20 min. circulation of a 0·2% solution of 'Calgon' (sodium hexametaphosphate) in softened water at 75° C.: addition of caustic soda to give a 0·5% concentration and circulating for a further 20 min.: flushing with hot and cold water. A 0·5% Calgon solution is recommended where only hard water is available. Bergum(73) has confirmed the value of Calgon, but Honoré(74) still favours circulating solutions of washing soda (0·5% at 80° C.) or preferably caustic soda.

The deterioration of H.T.S.T. and holder-pasteurized milk has been studied by Ashton(75) by (*a*) colony counts and coliform tests, (*b*) methylene-blue and resazurin tests, (*c*) keeping quality at 18° C. to end-point of 68% alcohol and clot-on-boiling tests. Most authors(76, 77, 78) favour the phosphatase, colony count, coliform and methylene-blue reduction tests for the bacteriological grading of heat-treated milk, and in general report better results from dairies using H.T.S.T. plant than from those with holder plant. That psychrophilic bacteria do not survive pasteurization temperatures has been confirmed by Chandrasekhar(79), and Rogick & Burgwald(80). Egdell & Bird have also studied the bacteriology of commercially pasteurized milk(81), and have investigated the types of bacteria present(82) by plating on Yeastrel milk agar incubated at 22, 30 and 37° C. Of the total at 22° C. aerobic spore-forming rods represented 9·6%, microbacteria 38·8%, Gram-negative rods and coccobacilli 5·0%, streptococci 24·3% and micrococci 21·7%. At 37° C. the corresponding figures were 16·2, 2·4, 5·2, 48·1 and 28·1%. *Micr. lacticum* and *Str. thermophilus* predominated among the microbacteria and streptococci respectively. *Str. lactis* and *B. cereus* predominated in thirty-seven cultures isolated from forty milk samples rejected by the keeping quality test. The influence of unsatisfactory raw milk on the keeping quality of pasteurized milk is reviewed by Anderson & Meanwell(83), and these authors, with Wright(84), have described a new test suitable for plant control purposes: the milk sample is incubated at 26° C. for 24 hr., and to 1 ml. is added 1 ml. of 68% alcohol coloured with methyl red: samples were classified as follows: grade 1, no change = acidity of 0·185% as lactic acid; grade 2, precipitation and no colour change (acidity < 0·28%); grade 3, precipitation and colour change (acidity > 0·28%).

With regard to coliform organisms and pasteurization, Gilcreas & O'Brien(85) have published interesting figures on the time/temperature relationships for the destruction of coliform organisms in the H.T.S.T. process. The mean times expressed in seconds for 99·99% destruction of a 'heat-resistant' strain of *Bact. coli* isolated from pasteurized milk were: 318 at 62° C., 83 at 65° C., 55 at 66° C., 15 at 68° C., 7·0 at 70° C., 5·4 at 71° C., 3·9 at 72° C. and 2·0 at 75° C. It appeared that the number of survivors was a function of time and temperature approximately logarithmic with respect to time over at least a part of the range. Several other workers(86, 87) have studied coliform bacteria in milk, and their resistance to pasteurization, and Wilssens(88) has typed 252 strains from 116 samples of pasteurized milk by means of biochemical and physiological tests: although most types were encountered, *Bact. coli* type II, *Bact. coli* irregular type V and *Bact. aerogenes* type I predominated. The serological approach to such studies is to be recommended; the colibacteria are serologically relatively complex organisms and a correlation of antigenic structure and heat resistance would be of interest not only in the field of pasteurization.

The importance of *Rickettsia burneti* has been appreciated(89), and Marmion, MacCullum, Rowlands & Thiel have determined the requirements for its destruction in naturally and artificially infected milk by commercial H.T.S.T. and laboratory holder pasteurizers. Post-pasteurization milk samples were examined by the phosphatase test, and by guinea-pig inoculation for *R. burneti*. With commercial plant and naturally infected milks 154–6° F. for 15 sec. rendered the organisms non-infective for guinea-pigs, but with artificially infected milk containing large numbers of *R. burneti* 161° F. was necessary. In the laboratory pasteurizer 143·5–144° F. for 30 min. destroyed *R. burneti*, but not 142·7° F. Rowlands(90) has discussed the time/tempera-

ture relationships commonly used in pasteurization with particular reference to their effect on the tubercle bacillus. The incidence of *M. tuberculosis* in raw and pasteurized milk has been studied by Wagener & Eberhardt(91) in Germany, and, according to Reilly(92), the decline during the last 10 years in human tuberculosis of bovine type in Northern Ireland can be attributed directly to the great increase in the consumption of pasteurized milk during that period.

Other processes

Burton(93) has discussed the possible means, other than true pasteurization, of destroying bacteria in milk. Two recent historical surveys cover the milk sterilization process(94, 95), and some modern systems are described; the effects on bacteria and nutritive value are discussed in the latter. Aschaffenburg(96) has described a simple ammonium sulphate turbidity test for sterilized milk based on the denaturation of milk protein (primarily albumin) by heat. It is worth making the comparison here with his zinc sulphate turbidity test for 'immune lactoglobulins'(97) in the serum of new born calves which is based on the interaction of the zinc ion with globulin (not albumin) present. Martin(98) described a process in which fresh milk is sterilized in a continuous heat exchanger and immediately canned aseptically in an atmosphere of steam: the resulting product could be kept 6–9 months without refrigeration. A pilot plant has also been described(99) for the rapid heat processing of fluid foods by steam injection. Papers have appeared on condensed milk by Hawley(100), Pearson & Nelson(101), Bothe(102) and Kreveld(103). It has been recommended(104) that homogenized milk shall be frozen as soon as possible after processing, but if the milk is of good quality, it may be kept at 35° F. for as long as 5 days before freezing, without adverse effect on the keeping quality of the frozen product: further(105), frozen homogenized milk of good quality may be held at normal storage temperatures after thawing, for longer periods than it is usually necessary to hold fluid milk before use. Antoniani & Cerutti(106) report the churning of normal butters from milk stored at −40° C. for 420 days, or −25° C. for 270 days. 'Pasteurization' of milk by means of high frequency electrical energy has been studied by Wiechers, de Zeeuw & van den Bosch(107) who found that reduction of bacterial count depended on the final temperature reached by the milk during treatment. No specific non-thermal effect was found by other workers(108, 109) in this field, except Lösche & Mücke(110); they found a fluctuation in bacterial content over the increasing time/temperature range, and considered the drop in bacterial numbers to be specific in the first part of the range, and a second decline later (an increase intervened) to be purely thermal. Nyrop(111) combines high-frequency electrical energy with ultrasonic waves, and describes a process for the pasteurization of milk in which these are applied simultaneously; the pasteurization time was 35 sec. and no cooling was necessary. Davis(112) has considered ultrasonics and other alternatives to the pasteurization of milk. Springer & Köchl(113) achieved limited success with ultrasonic waves, but estimate that in the event of complete sterilization of milk by these means vitamins B_2 and C would be completely destroyed. Leali(114), Stucki(115) and Voogd & Daams(116) have studied the effect of ultra-violet light on bacteria; good results have been reported in this connexion(117) and for the keeping quality of irradiated milk(118). An interesting description(119) has been given of a new stainless steel ultra-violet milk irradiation plant of 3000–6000 l./hr. capacity. American workers at the Institute of Technology, Cambridge, Massachusetts, have made intensive studies(120, 121) of the effect of X-rays on bacteria and on bacteria in milk: *Staph. aureus* proved to be more radio-resistant than *Bact. coli*, *Serr. marcescens*, *Ps. aeruginosa* or *Ps. fluorescens*. Most bacteria were destroyed by X-rays with a dose of less than 500,000 Röntgens, but spore formers required $1{\cdot}5 \times 10^6$ Röntgens or more. Milk required 10^6 Röntgens for complete sterility. Irradiation with high voltage cathode rays reduced the bacterial content of milk from 37×10^6 to 2 per ml. Cheese produced from irradiated milk appeared normal, but irradiation of butter destroyed its colour. Burton(122) has discussed the sterilization of milk by radiations. De Vleeschauwer, Hendrickx & Naudts(123) report unfavourably on the use of oxygen in the pasteurization of milk. The subject of antibiotics has been further dealt with elsewhere in

this Review (p. 386), but several papers treat especially of the preservation of milk by these substances: Greene & Bell(124) have dealt with various antibiotics, and in general, variable results are reported, both for numbers and types of bacteria(125, 126, 127). A combination of heat and antibiotic treatment has been used by Anderson & Michener(128) for the successful preservation of a number of foods, including milk, against microbial spoilage: mild heat was used to destroy vegetative cells, yeasts, fungi and enzymes, and subtilin, or other antibiotics, with heat to destroy spores. The preservation of human milk with antibiotics has also been studied(129, 130, 131).

The use of hydrogen peroxide and formaldehyde as preservatives for milk received attention in some countries during the war. At one time (1945–6) H_2O_2 was used extensively in France, and Pien(132) recommends the addition, at the farm, of 1–2 ml. of 130 vol. hydrogen peroxide per litre of milk. Good results have been obtained with H_2O_2(133, 134, 135), especially at a concentration of 300 p.p.m.(136), without adverse effect on the nutritive constituents of the milk, though other workers(137) do not favour its use because of undesirable effects on the colour and taste of milk. Nambudripad & Iya(138) have shown, in confirmation of earlier work, that catalase-producing organisms are usually readily destroyed by hydrogen peroxide, but the spores of aerobic spore-forming organisms are very resistant to its action. Hydrogen peroxide treatment is recommended by Giolitti(139) only for tubercle-free milk, since he found that *M. tuberculosis* resisted concentrations as high as 2%; he recommends large additions to milk, with subsequent elimination of the residual H_2O_2 by catalase from bovine liver(140). Morris(133) has studied cheese made from peroxide-treated milk; the cheese had a much higher water content than normal, resulting in a pasty texture and soft body. Monaci(141) and Biffi & Romagnoli(142) have examined the effect of hydrogen peroxide on a number of pathogens and have obtained satisfactory results with concentrations which would normally be used in practice. A method for canning whole milk has been described(143) in which, prior to the actual canning process, H_2O_2 (1% by weight) is added to the milk and allowed to act for 30 min. The recommended concentration of formaldehyde for the preservation of milk and improvement of its keeping quality is 0·1 g./l.(144, 145), subsequently neutralized with sodium or potassium bisulphite(145). However, undesirable flavours of various kinds were induced in the milk(145), and it is concluded that the method does not solve the sour milk problem. Tests for the presence of formaldehyde in milk are considered in two papers(146, 147), one (147) of a comprehensive nature. Fidler(148) advocates the use of furacin (1:25,000) as a preservative for fresh milk since it was active against proteolytic organisms and had no adverse effect on the colour or flavour of the milk.

Dried-milk powder

A comprehensive paper(149) from Holland gives in detail the Dutch regulations concerning dried milk: physical and chemical requirements are given as well as standards for sampling and inspection, and of bacterial count and keeping quality: the Dutch recognize four grades of dried milk, and the requirements for each are specified. The importance of the temperature of reconstitution of milk powder has now been accepted, and White(150) has recently confirmed the original observations of Hiscox(151) and others that samples of spray- and roller-dried milk powder gave higher plate counts when reconstituted at 50° C. than when reconstituted at room temperature. Lithium hydroxide is lethal to the organisms generally occurring in dried milk, and contact for 15 min. with a 0·1 N solution may destroy 99–100%. Reconstitution at 40° C., or less, may thus lead to errors in grading which would be multiplied if 0·1 N-lithium hydroxide were used as the reconstituting medium. The introduction in England in 1943, in the drying process, of preheating at 190° F. has been found to be of considerable value from the bacteriological point of view by Higginbottom(152), who has continued her work on dried milk. After the introduction of the high preheating temperature there was a definite decrease in the number of organisms surviving in the powders tested, and a lower standard deviation among counts indicating a bacteriologically more uniform product. Some authors(153), however, still report considerable variation among samples from the different sources of National Dried Milk.

Higginbottom(154) has also studied the types of bacteria occurring in spray-dried milk, *Str. faecalis* and *Micr. casei liquefaciens* (Orla Jensen) predominating: *Str. lactis*, *Str. thermophilus* and spore-forming bacilli were found less commonly. In view of the absence of staphylococci and the lack of evidence implicating *Str. faecalis*, or the spore-formers isolated, in enterotoxic poisoning, this danger from dried milk is probably slight. However, Higginbottom has made the important observation that reconstituted milks aged at 15·5° C. for 24 hr. remained virtually unchanged in flora and total count, whereas samples aged at 22° C. showed a 2000-fold increase in mean total count, with the same main types of bacteria present but a considerable increase in the number and variety of spore-forming bacilli. The effect of different relative humidities on the bacteriology of milk powder has also been studied by the same author(155), who exposed two samples to R.H.'s of 0–100% at 25° C. for 48 and 103 weeks: she found, in general, that the greatest antibacterial effect was obtained towards either extreme of the R.H. range. At R.H.'s above 70% there was a rapid reduction in count within 2–4 days, followed by the growth of bacteria and moulds as the moisture content increased. After storage for 48 weeks at 0% R.H. the total number of bacteria present had been reduced by 94%.

Ice-cream

Although they do not approach the question from a bacteriological point of view, two new books deserve the attention of those interested in ice-cream. One, by Frandsen & Nelson(156), deals with all aspects of the manufacture and properties of ice-cream and allied frozen commodities, while the other, *Refrigeration Principles and Practice*(157), reviews the present knowledge and position of refrigeration: dairy products are considered, and there is a short chapter on ice-cream. General papers on ice-cream have been written by Ostertag(158), who presents a comprehensive review of the world literature on the control of bacteriological quality, and by Arbuckle(159), who gives a wealth of general information on the subject including ingredients, pasteurization, storage and defects. A synopsis(160) has been prepared by the International Association of Ice-Cream Manufacturers on the proposed regulations of the Food and Drug Administration, U.S.A., on standards for ice-cream and related foods. Reid, Agee, Hanckel & Thomas(161) have added to ice-cream mix 3·5% of a dehydrated skim-milk culture of lactic acid bacteria; the resulting product had an agreeable flavour and its texture and stability were superior to those of normal mixes.

G. S. Wilson(162) has summed up the effect of the Ice-Cream (Heat Treatment) Regulations, 1947, and the present position of sampling and grading ice-cream, and has made suggestions for improvement in plant supervision and sampling. The bacteriology of ice-cream at different stages during processing has been investigated(163, 164), and it has been shown that colony counts increase progressively during manufacture: in the samples examined(163), the colony counts increased from 10,000 to 15,000/ml. in the mix before freezing, to about 26,000–28,000 in the finished product. Pipes, pumps, freezing apparatus, cutting knives and air in the hardening tunnel were the main sources of infection. The third and fourth reports(165, 166) of a Sub-Committee inquiring into tests for the bacteriological grading of ice-cream have been published, and the committee's work—primarily on the methylene-blue test—has been concluded. It is recommended(166) that grade I samples be defined as those samples which do not reduce methylene blue completely in 4 hr. The presumptive coliform test has not been recommended, since it overestimated the number of samples containing 'faecal coli', while the majority of these were not *Bact. coli* (I), but irregular types. However, Patton(167) has recorded general agreement between methylene blue, colony count and coli-aerogenes tests, and recommends provisional standards for statutory control as follows: (*a*) ice-cream should not contain more than 200,000 organisms/g., (*b*) coli-aerogenes bacteria should be absent in 1 g. Recently, Schade(168) has also discussed the incidence and source of coliforms in ice-cream. Rosati(169) has suggested yet another scale of judgement; namely, ice-cream should contain less than 500,000 bacteria, and less than 10 coliforms, per ml. Approximately two-thirds of the ninety

samples he examined from five different sources attained these limits; five samples contained enterotoxic staphylococci.

Hobbs(170) and Borneff(171) have also outlined methods for the bacteriological examination of ice-cream and Hobbs(170) has also given the results of grading by the methylene-blue test during 1948–50. The survival of viruses in ice-cream has been studied, the Lansing strain of poliomyelitis virus in particular, and it has been shown that(172), in ice-cream, it survived for 5 months at 4° C. and for 4 months at room temperature; these figures were higher than those obtained when the virus was suspended in saline. Moreover, higher temperatures of heat treatment were tolerated in ice-cream than in saline.

Tobias & Tracy(173) observed no major differences in the quality of ice-cream pasteurized by either the H.T.S.T. or holder methods, and the effects of the two processes on the bacterial flora were similar. Concerning H.T.S.T. pasteurization, Tracy, Tobias & Ordal(174) report the best results with a time/temperature combination of 177·5° F./30 sec. This is greater (in both time *and* temperature) than most workers recommend, but it is stated that mix treated in this way compared favourably with that heated at 160° F. for 30 min., in respect both of bacterial destruction and flavour. A 25 sec. heat treatment at 175° F. is most commonly recommended(175, 176, 177) for destruction of both the normal bacterial flora and heat-resistant *Micrococcus*(176, 177) and *Microbacterium*(177) species. It is uncertain whether the theoretical danger of using heat-resistant substitutes for *M. tuberculosis* (see p. 408) in the assessment of pasteurizing efficiency is greater in ice-cream than in milk, but this seems, at least, to be a possibility. Tracy and his colleagues(178, 179) have studied the efficiency of the vacreator and mallorizer used to pasteurize (H.T.S.T.) ice-cream mix and have concluded that the practicable range of temperatures for these appliances is 185–240° F. The viscosity of the mix increased with increasing temperature, contrary to Arbuckle & Nisonger's(180) findings with simple H.T.S.T. pasteurization. The determination of the holding times of plants pasteurizing ice-cream mix has been discussed by Botham(181), and a comprehensive paper by Thiel & Burton(182, 183) reviews the factors which influence the flow and holding time of ice-cream mixes. As they point out, the viscosity of the mix varies with its composition, and holding time is affected by viscosity; it is essential, therefore, that H.T.S.T. plant be designed and operated in such a manner that changes in viscosity can never result in a holding time of less than 15 sec., and that the rate of flow through the holder remains constant.

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